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Supplementary Materials for

Adjuvant-free nanofiber vaccine induces in situ lung dendritic cell activation and T_H17 responses

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This PDF file includes:

Supplementary materials

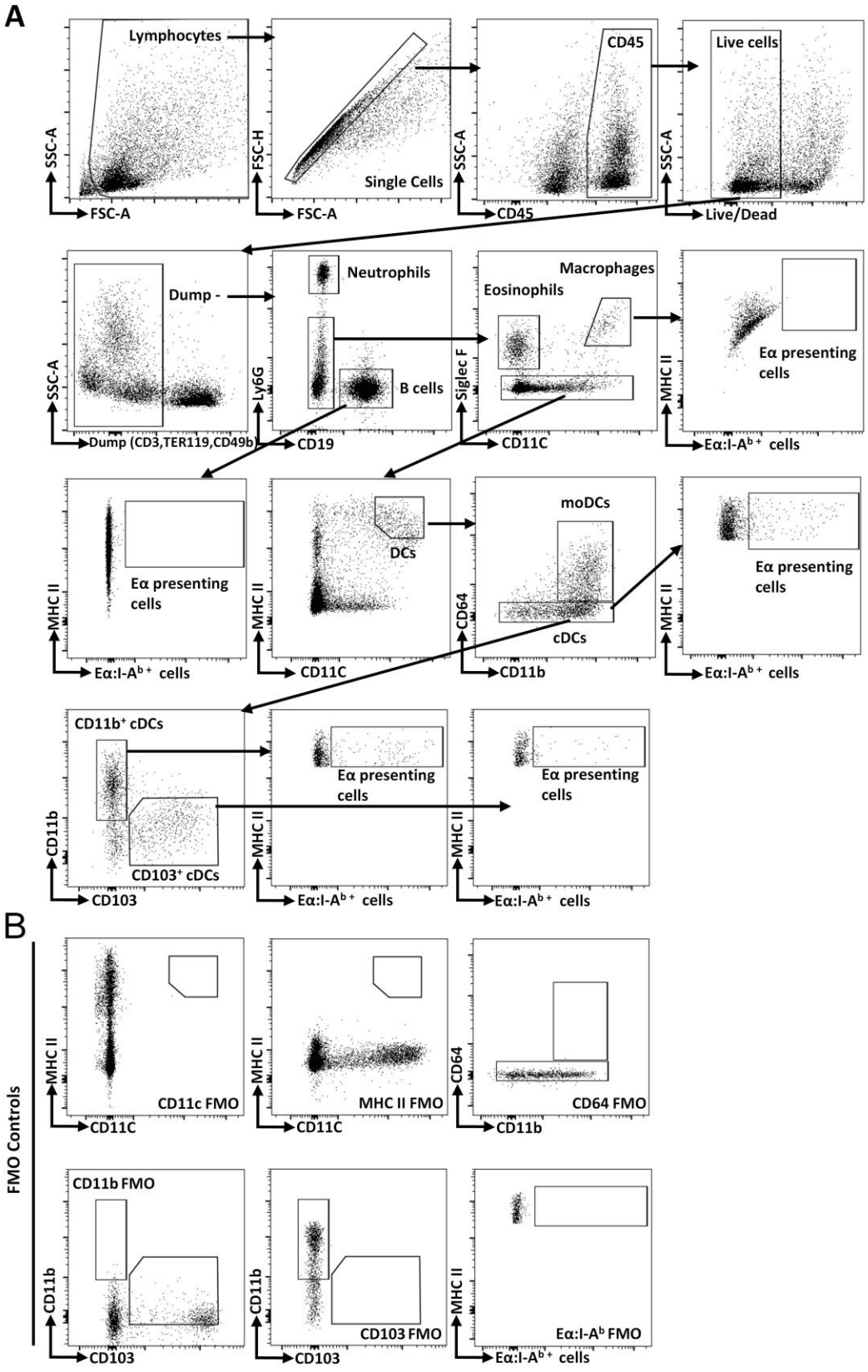

Fig. S1. Flow cytometry discrimination of lung cell populations. (A) Gating strategy for the identification lung DC and DC subsets. (B) FMO controls.

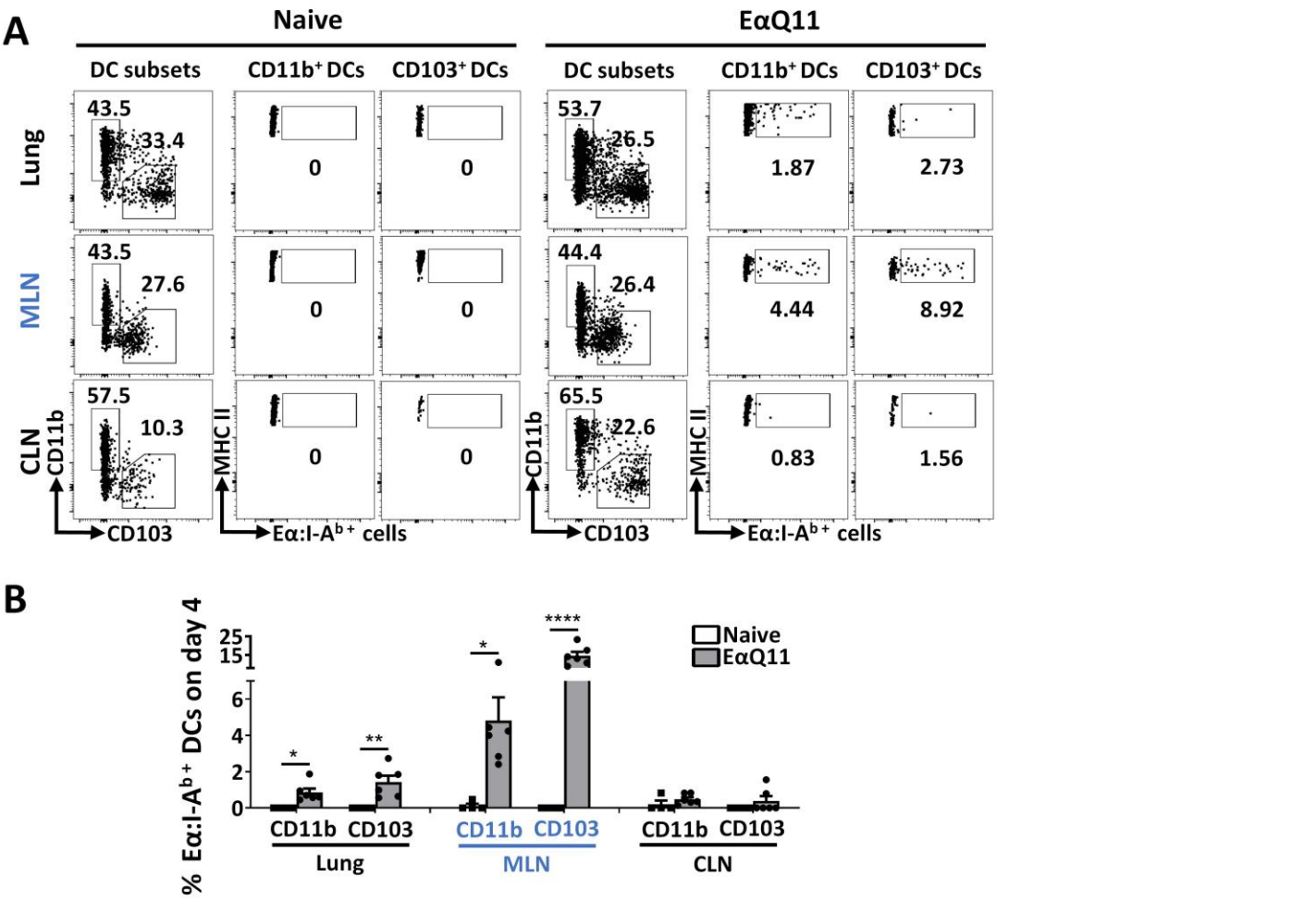

Fig. S2. Eα presentation by DCs in lung, mediastinal (MLN) or cervical (CLN) lymph nodes on day 4 after EαQ11 I.N. immunization. C57BL/6 mice were intranasally immunized with EαQ11. Lung, MLN and CLN were collected on day 4 post-immunization. (**A**) Representative flow cytometry plots displaying pEα:I-A^b-positive $CD11b^+$ or $CD103^+$ DCs in the lung, MLN and CLN. (**B**) Percentage of Eα:I-A^b-positive $CD11b^+$ or $CD103^+$ DCs in the lung, MLN and CLN. Each dot represents 1 mouse. Data are mean ± SEM from 2 independent experiments. ***p< 0.001, **p< 0.01, *p< 0.05 by two-way ANOVA.

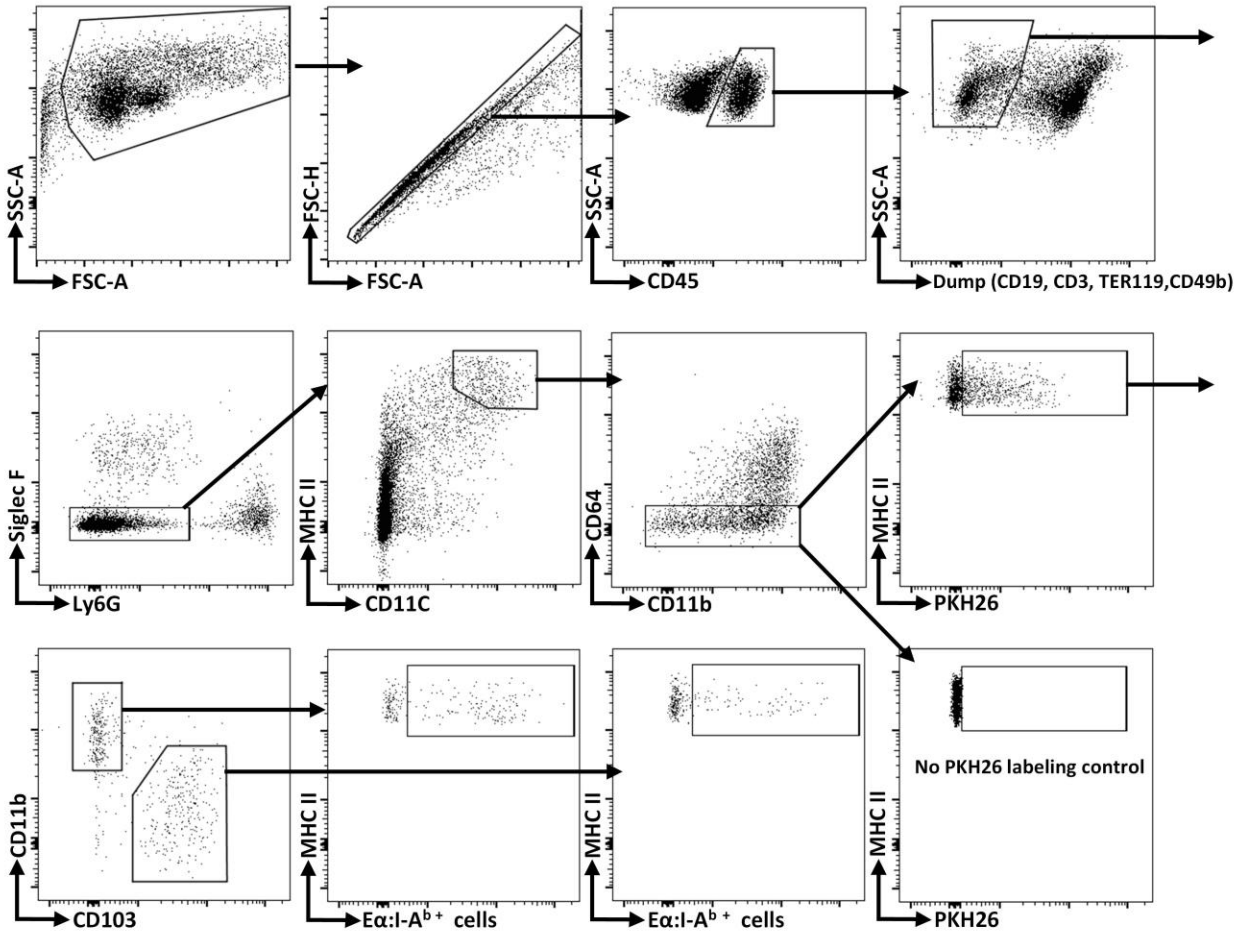

Fig. S3. Gating strategy for the identification PKH26^{+} DC subsets in lung.

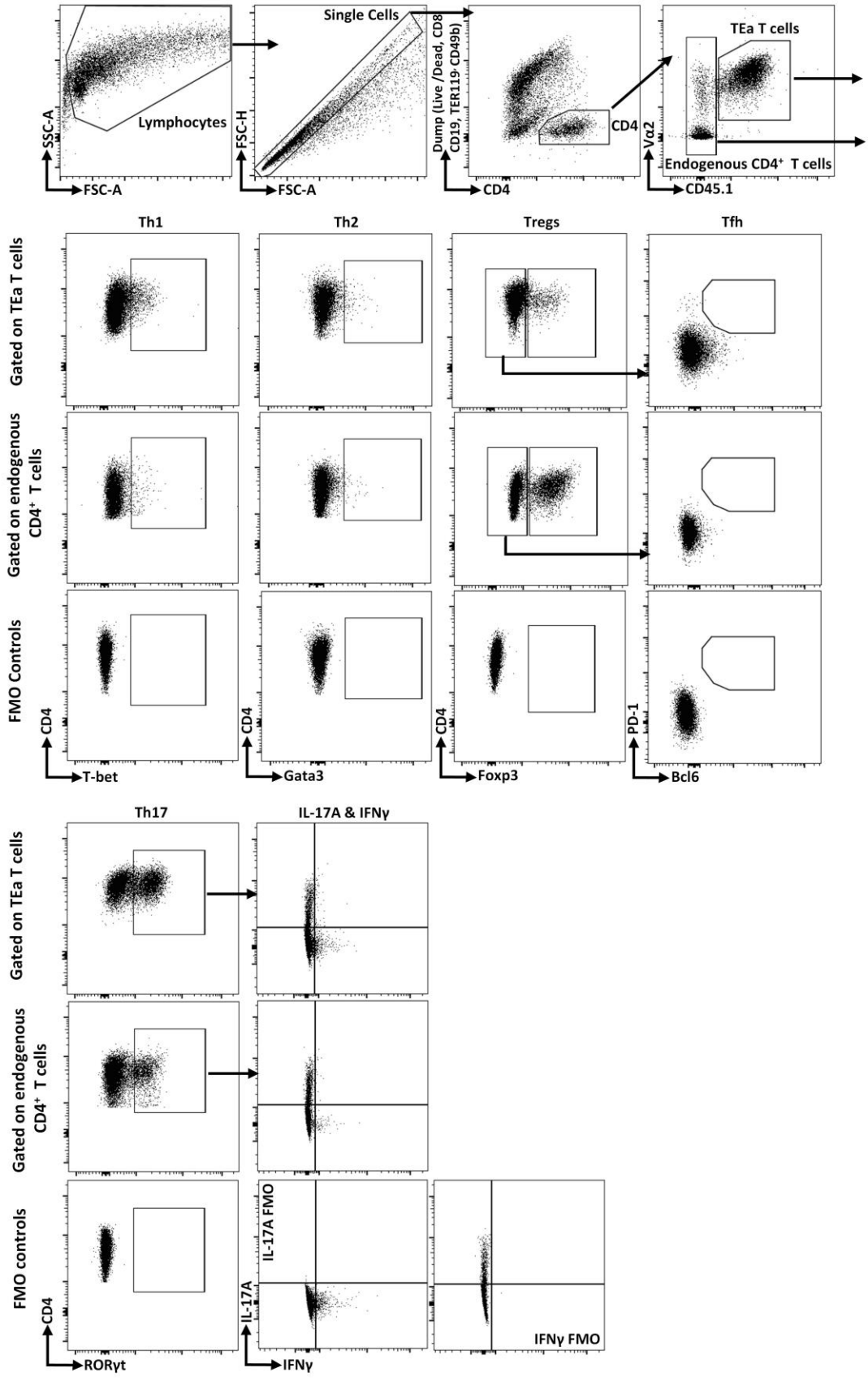

Fig. S4. Gating strategy for identifying Th1, Th2, Th17, Tregs, IL-17A or IFNγ producing RORγt+ cells in the lung.

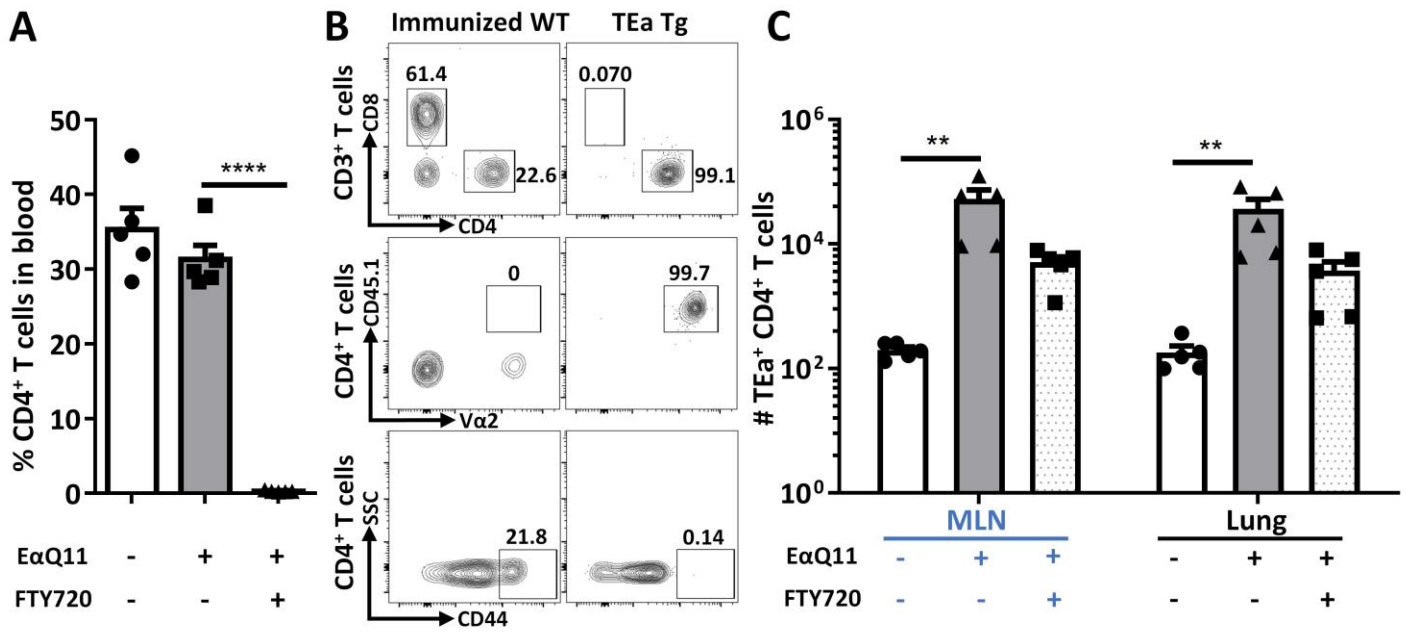

Fig. S5. Egress of lymphocyte from secondary lymphoid organs is blocked after FTY720 treatment. (**A**) Percentages of CD4⁺ T cells in the blood at 4 days after FTY720 treatment. (**B**) Representative flow cytometry plots displaying low CD44 expression by naïve TEa cells used in adoptive transfer, compared to EaQ11-immunized B/6 T cells. (**C**) The number of TEa T cells 5 days after adoptive transfer of 10,000 TEa T cells, immunization and FTY720 treatment. Data shown are means ± SEM from ≥2 independent experiments. ****p< 0.0001, **p< 0.01 by unpaired, two-tailed t-test (**A, C**).

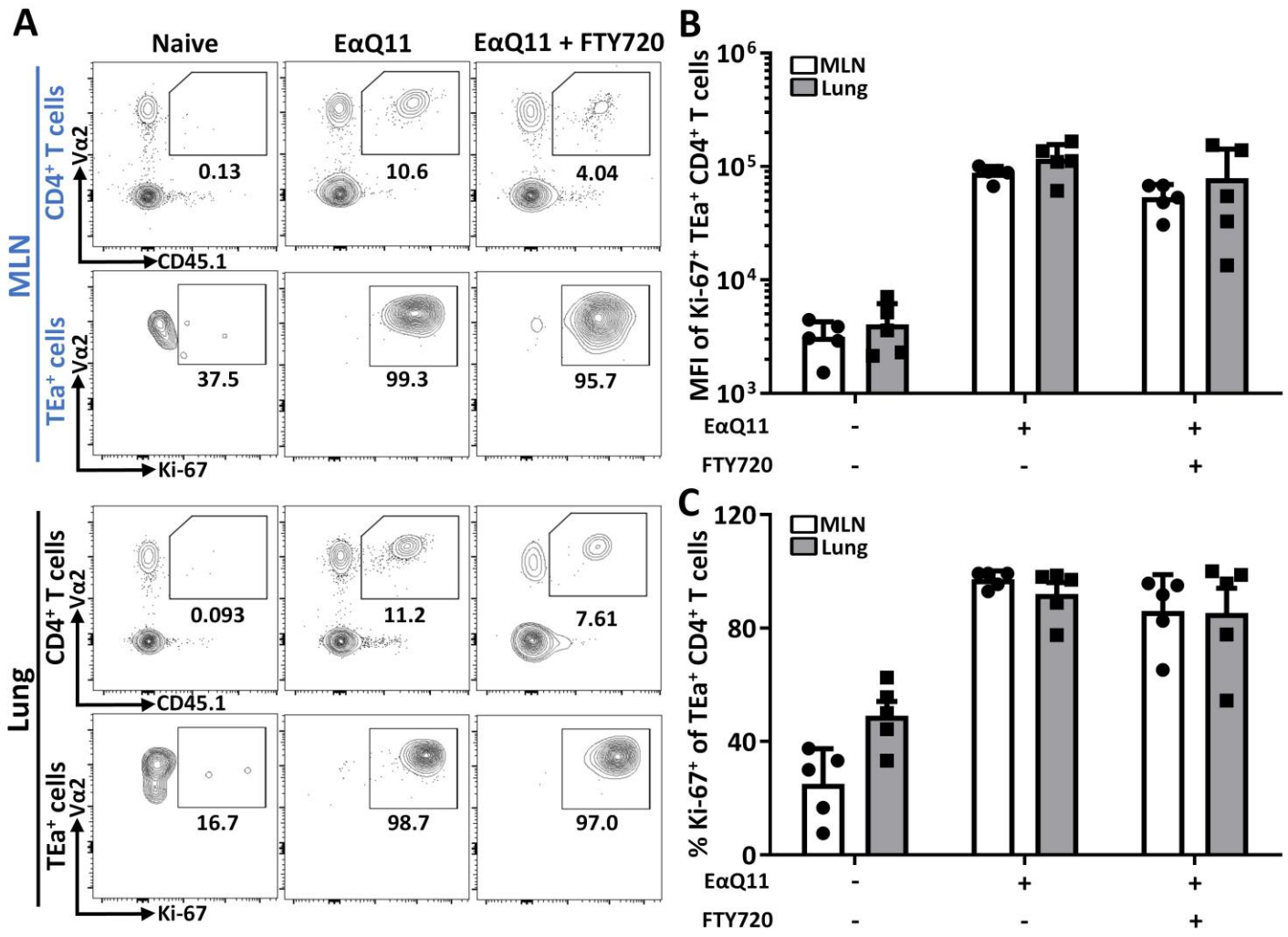

Fig. S6. Expression of Ki-67 by TEa T cells in the lung and draining LN. (**A**) Representative flow cytometry plots displaying Ki-67 expression by TEa T cells from the MLN (top) and lung (bottom), 5 days after adoptive transfer of 10,000 TEa T cells, immunization and FTY720 treatment. Data are presented as (B) MFI of Ki-67 expression or (**C**) percentage of Ki-67 positive of TEa T cells in the MLN and lung. Each dot represents 1 mouse. Data shown are means ± SEM from ≥2 independent experiments by unpaired, two-tailed t-test (**B, C**).

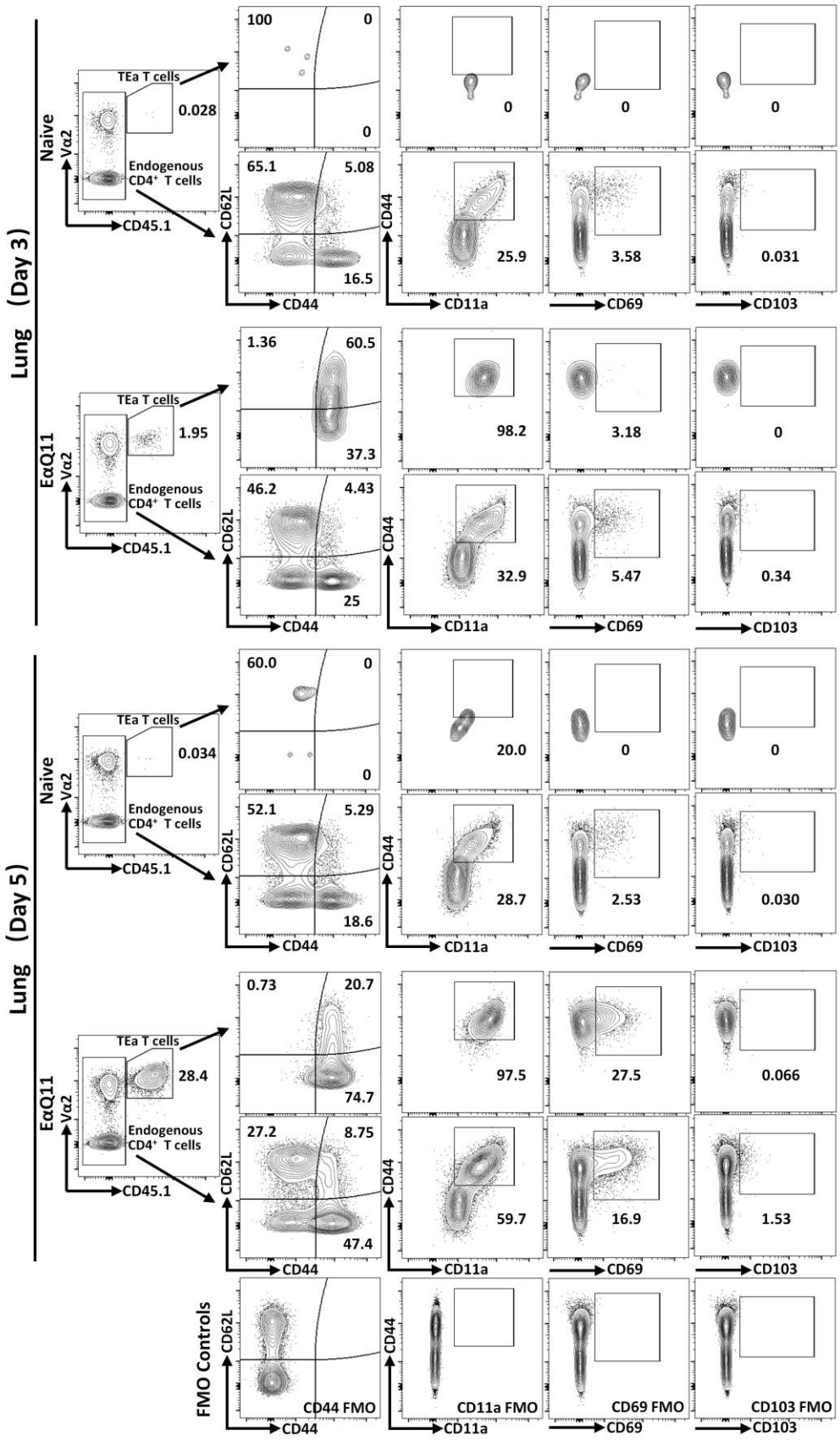

Fig. S7. Representative flow cytometry plots of the expression of CD44, CD62L, CD11a, CD69 and CD103 by lung TEa T cells.

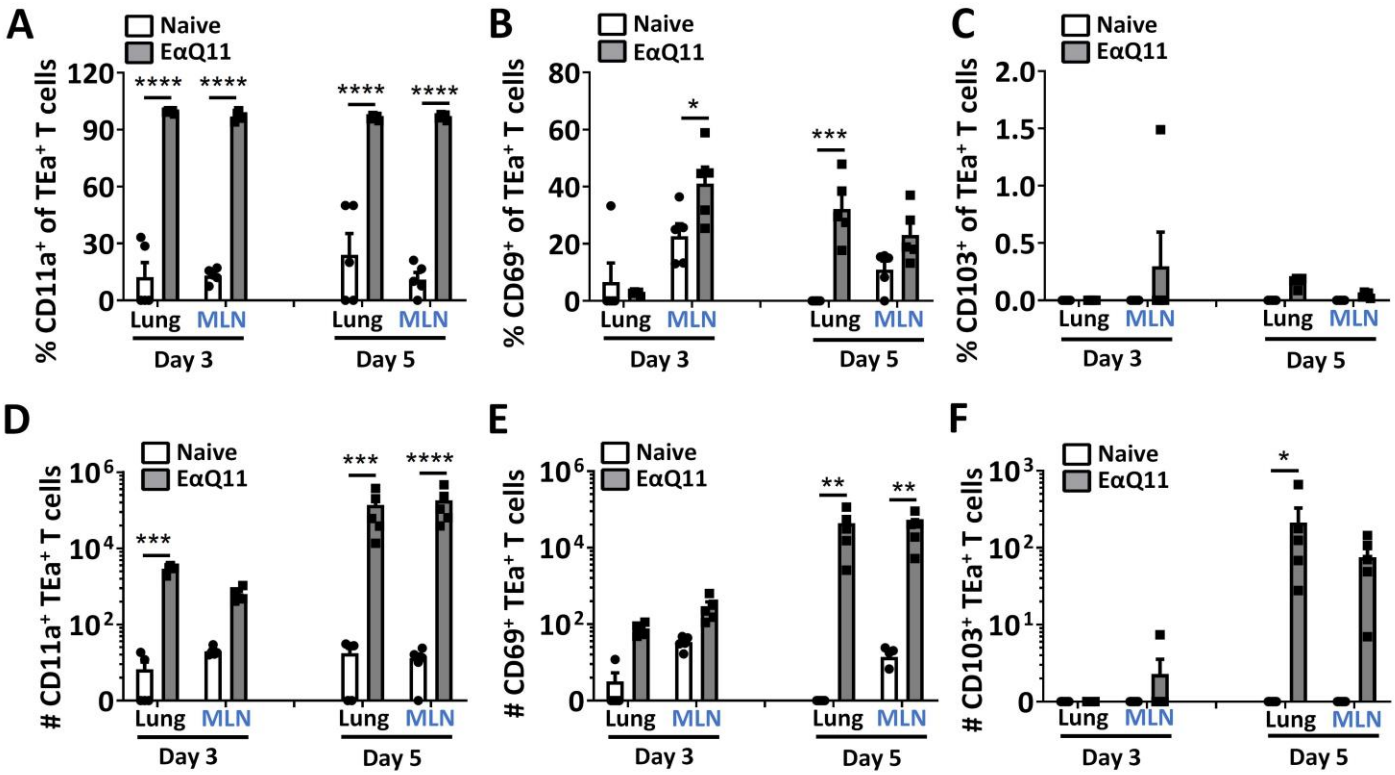

Fig. S8. Phenotype of expanded TEa T cell in lung and MLN on day 3 and 5 after adoptive transfer of 50,000 TEa T cells and EαQ11 I.N. immunization. (A) Percentage of CD11a+ TEa T cells in the lung and MLN. **(B)** Percentage of CD69+ TEa T cells in the lung and MLN. **(C)** Percentage of CD103+ TEa T cells in the lung and MLN. **(D)** The total numbers of CD11a+ TEa T cells in the lung and MLN. **(E)** The total numbers of CD69+ TEa T cells in the lung and MLN. **(F)** The total numbers of CD103+ TEa T cells in the lung and MLN. Each dot represents 1 mouse. Data shown are means ± SEM from ≥2 independent experiments. ****p< 0.0001, ***p< 0.001, **p< 0.01, *p< 0.05 by two-way ANOVA (**A-F**).