Supplemental material

Infectious dose (CFU)	Immune cells[a]	CFU recovered[b]	Health score[c]
1.5×10^9	N.A.	N.A.	3
3.0×10^8	8.4×10^4	4.3×10^8	2
6.0×10^7	4.2×10^5	1.5×10^{10}	1
1.2×10^7	1.7×10^5	2.1×10^6	0
6.0×10^6	9.6×10^4	2.5	0
Mock infected	3.5×10^4	0	0

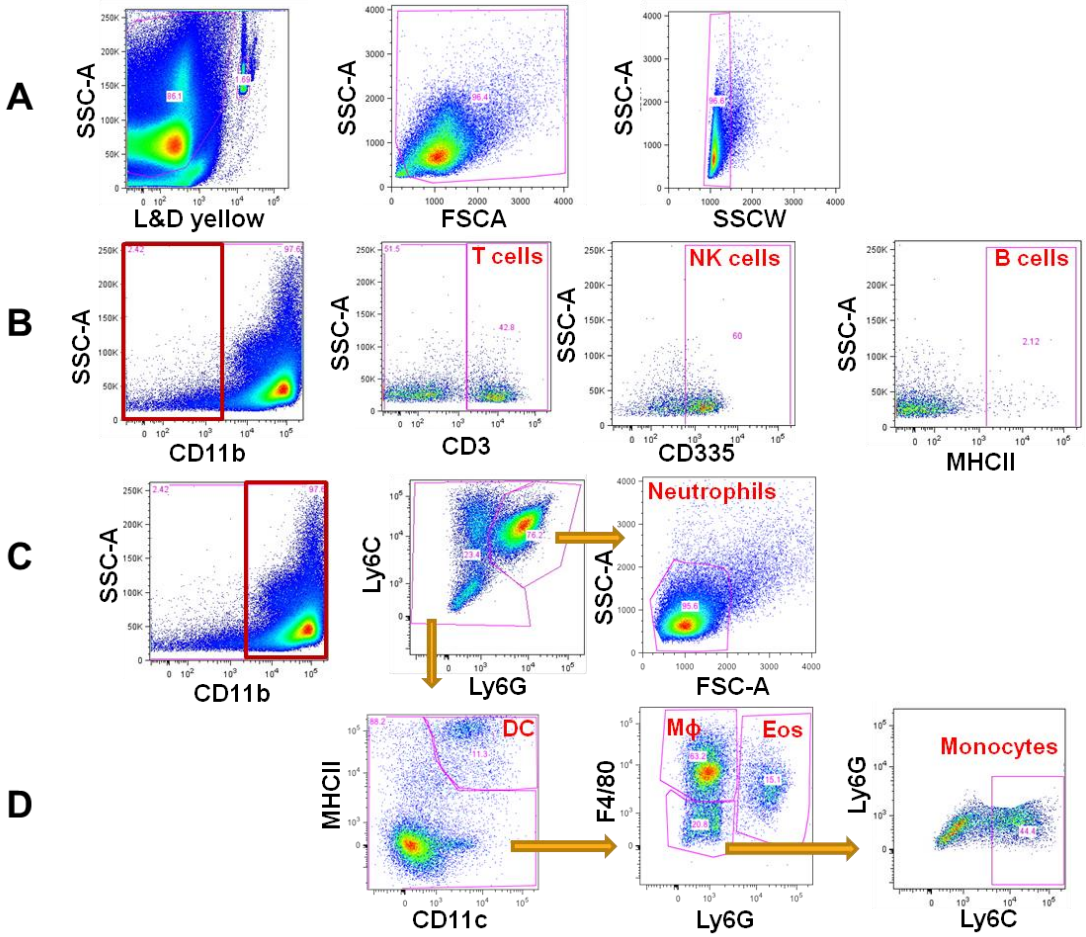
Supplemental Table S1. CFU and immune cells recovery from the pouch.

[a,b]Values of immune cells and CFUs recovered from the pouch are expressed as geometric mean of values obtained from individual animals (10)

[c]Health score was defined as follows: 0 - no symptoms, 1 - ruffled fur, 2 - ruffled fur and impaired movements, and 3 - death occurred within 24 h

Supplemental Figure S1.

FACS gating strategy for the analyses of immune cell populations. Cells from pouch lavages were incubated with live/dead discrimination marker (L/D Yellow) and further surface-stained with fluorochrome-conjugated antibodies against phenotypic markers (CD3, CD335, CD11b, CD11c, MHCII, Ly6C, and Ly6G). Cells were first gated on live, morphology and singlet (panel A). Lymphoid cell populations were identified within the CD11b^{low} gate as CD3+ T cells, CD335+ NK cells (panel B). Myeloid cell populations were identified within the CD11b^{hi} gate as Ly6C^{hi}Ly6G^{hi} neutrophils (panel C) among the non-neutrophils, DC were identified as CD11c^{hi}MHCII^{hi}, macrophages as F4/80+Ly6G-, eosinophils as F4/80^{int}Ly6G+, and monocytes as F4/80-Ly6G-Ly6C+ (panel D). Red numbers in dotplots are the percentages of parent gates.



Supplemental Figure S2.

Repertoire of immune cell populations. Pie chart illustrating and quantifying the immune cell populations recruited in the pouch of mice immunized with alum-and 4C-Staph vaccine and then infected.