TABLE S1 Primers used in this study

Primer	Sequence
NAT-F	AGCGGATAACAATTTCACACAGGA
NAT-R	CGCCAGGGTTTTCCCAGTCACGAC
CAP60up-F	TGGCACCACAACATCTGGGA
CAP60up-R	ACAACGTCGTGACTGGGAAAACCCTGGCGATCGGTGACGGATGAGGGAT
CAP60down-F	CTGTTTCCTGTGTGAAATTGTTATCCGCTCGGGCTTCTAATGCACAGGT
CAP60down-R	GAAATGATGGTCACTCGACA
CAP60up-NotI-F	AAAAGCGGCCGCGCACCACAACATCTGGGA
CAP60down-SacII-R	AAAACCGCGGGAAATGATGGTCACTCGACA
CAP60rt-F	ACTCAACAACGCCGCCATGG
NATcheck-F1	GTCACCAACGTCAACGCACC
CAP60-downR1	GGAGGCGGAGTTAAAAGCAA

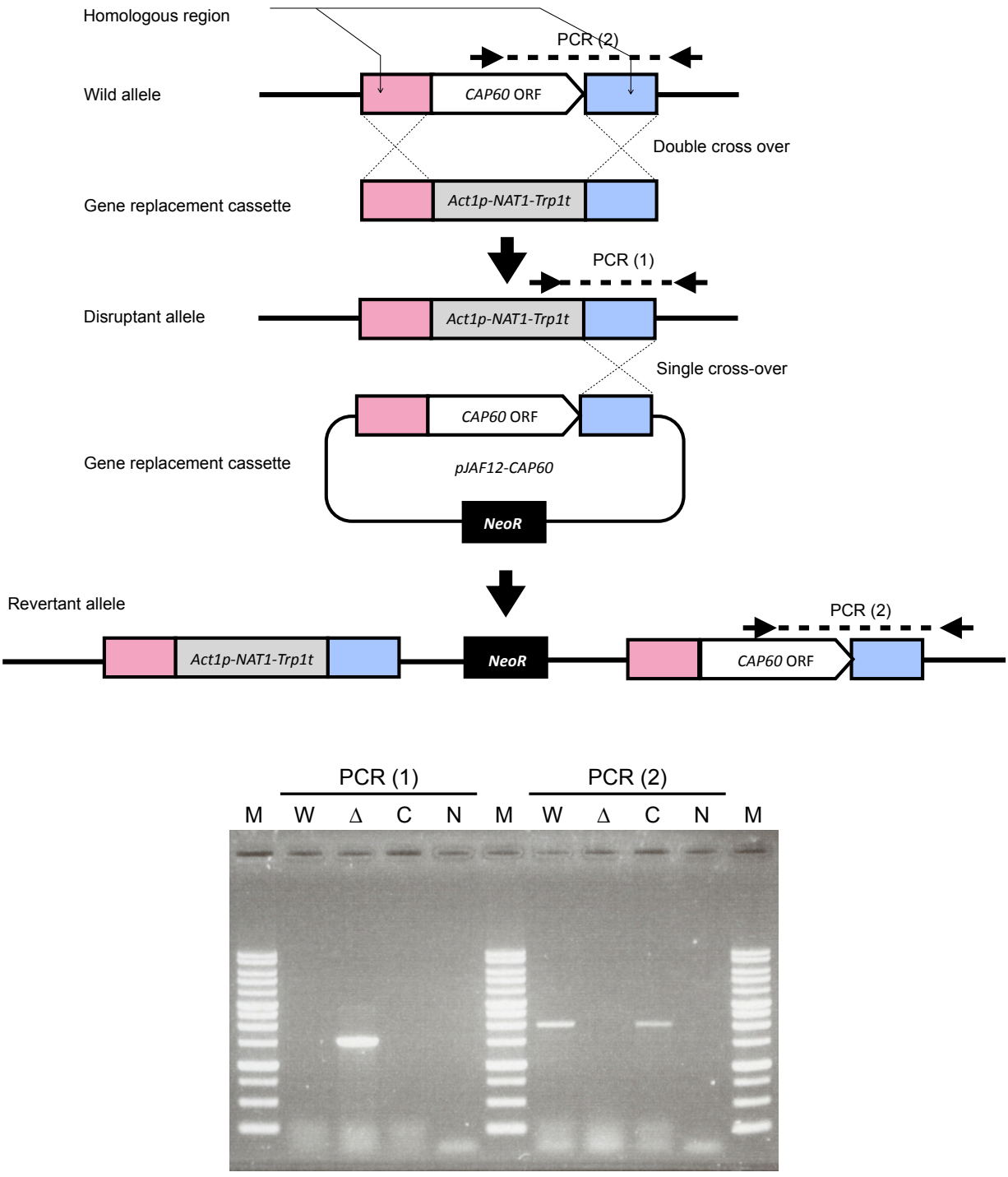

FIG S1 Gene disruption and reintegration. *CAP60* ORF, the region from the ATG start codon to the TAG stop codon, was completely deleted by homologous recombination. Deletant CAP60Δ was nourseothricin resistant, as it had nourseothricin resistant gene *NAT1* in its *CAP60* locus. Complement vector pJAF12-CAP60 was integrated into the *CAP60* locus of deletant CAP60Δ to generate revertant CAP60C. Because the complement vector harbored the geneticin resistant gene NeoR, revertant CAP60C was geneticin/nourseothricin double resistant. Two PCRs were used to confirm the intended recombination in transformants. The primers used for PCR were: (1) CAP60rt-F and Cap60-downR1 and (2) NATcheck-F1 and Cap60-downR1; M: 1 kb ladder, W: wild type strain PNG18, Δ: deletant CAP60Δ, C: revertant CAP60C, N: non-template control.

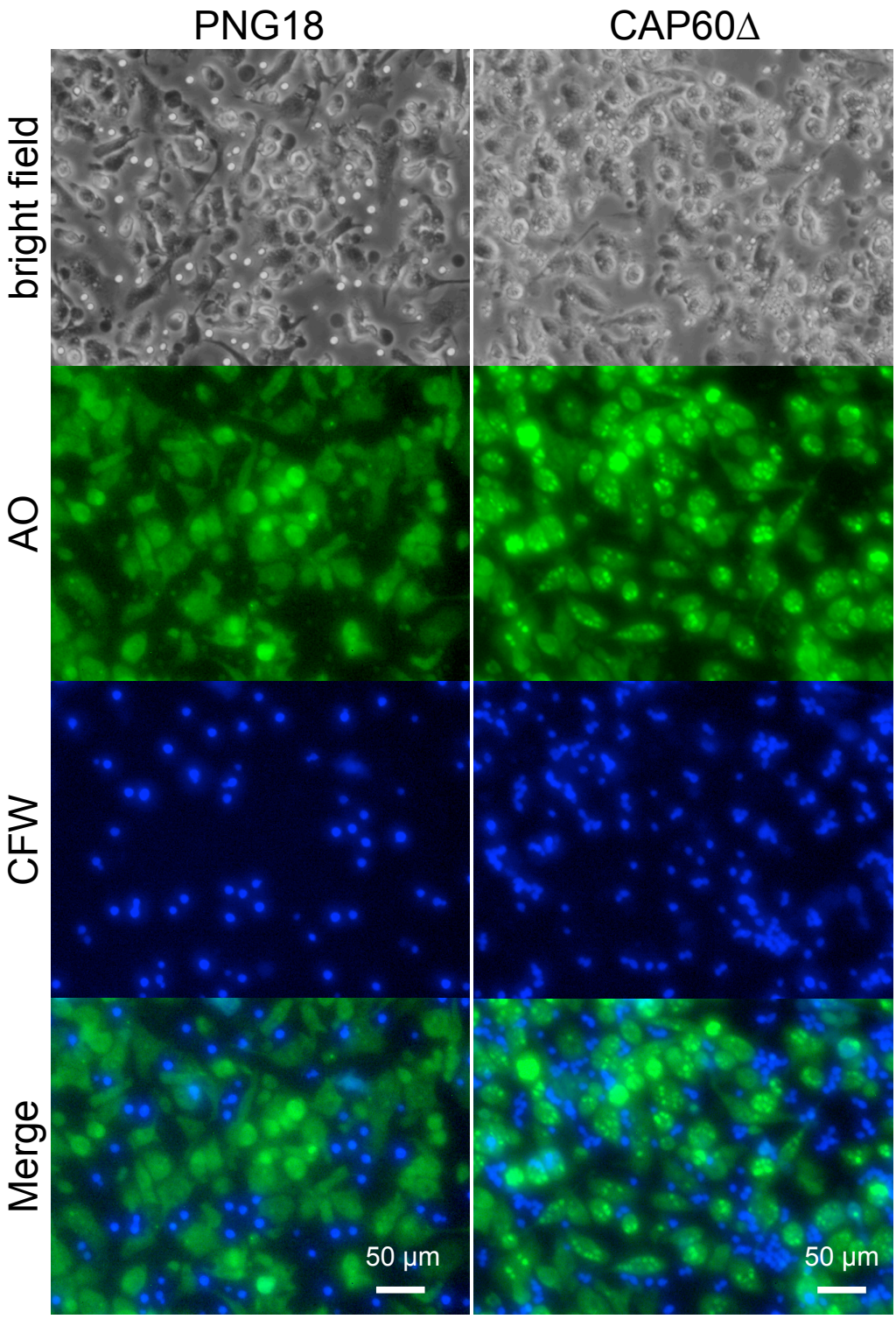

FIG S2 Several CAP60Δ cells are engulfed by each BMDC. Prior to incubation, heat-killed *C. gattii* cells were stained with acridine-orange (AO: green), which binds to nucleic acids. After 24 h incubation for phagocytosis, these cells were stained with calcofluor white (CFW: blue), which binds to chitin in the cell wall. Because this fluorescent dye did not enter BMDCs, engulfed fungal cells were not stained by calcofluor white.

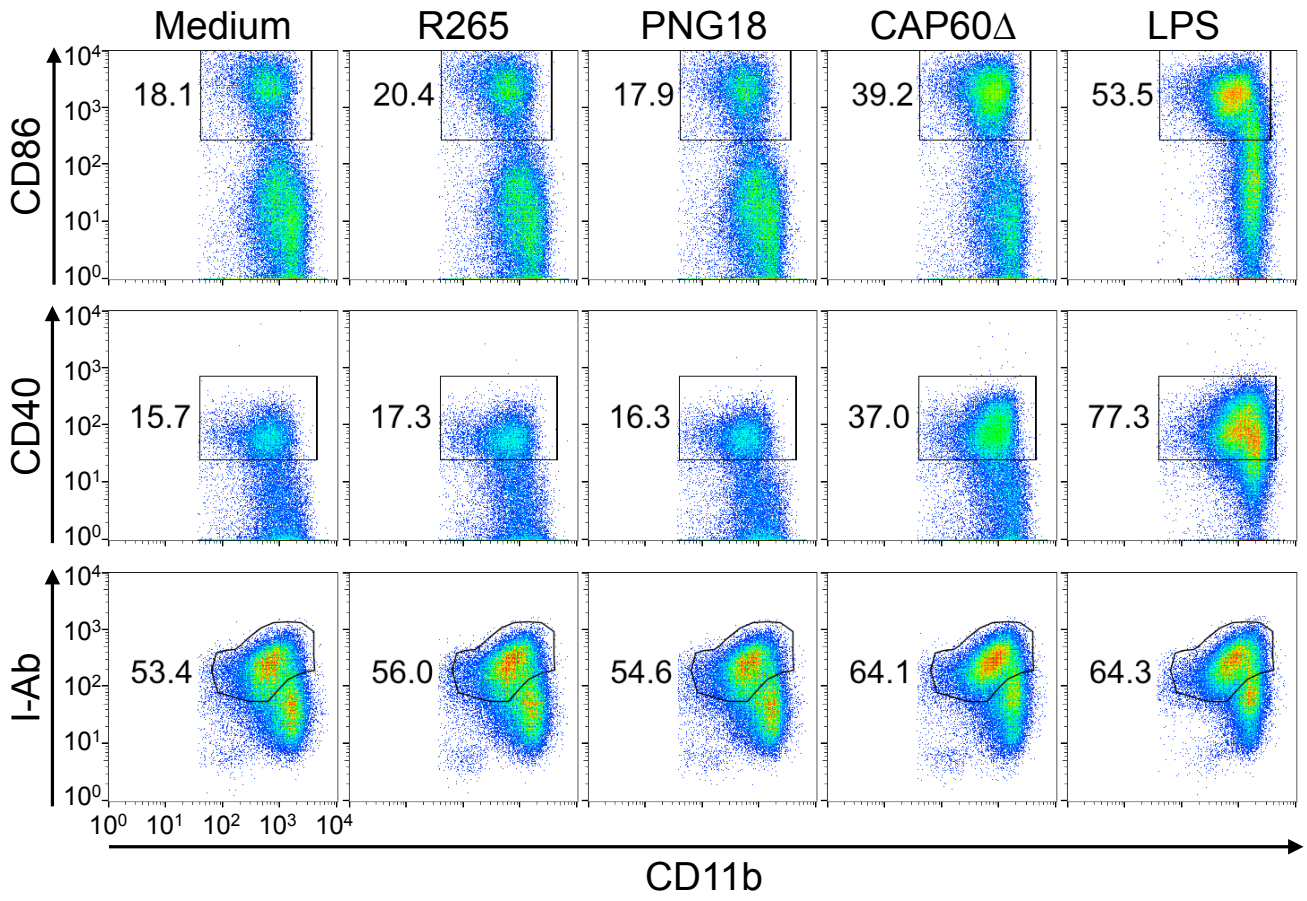

FIG S3 CAP60Δ induces BMDCs to express co-stimulatory molecules. BMDCs (2×10^6 cells/ml) were incubated with heat-killed strain R265 cells, CAP60Δ cells (MOI = 0.1), or 100 ng/ml of LPS for 24 h. BMDCs were collected and stained for CD11c, CD11b, CD86, CD40, and I-Ab. For flow cytometry analysis, gates were set for CD11c+ CD11b+ cells.

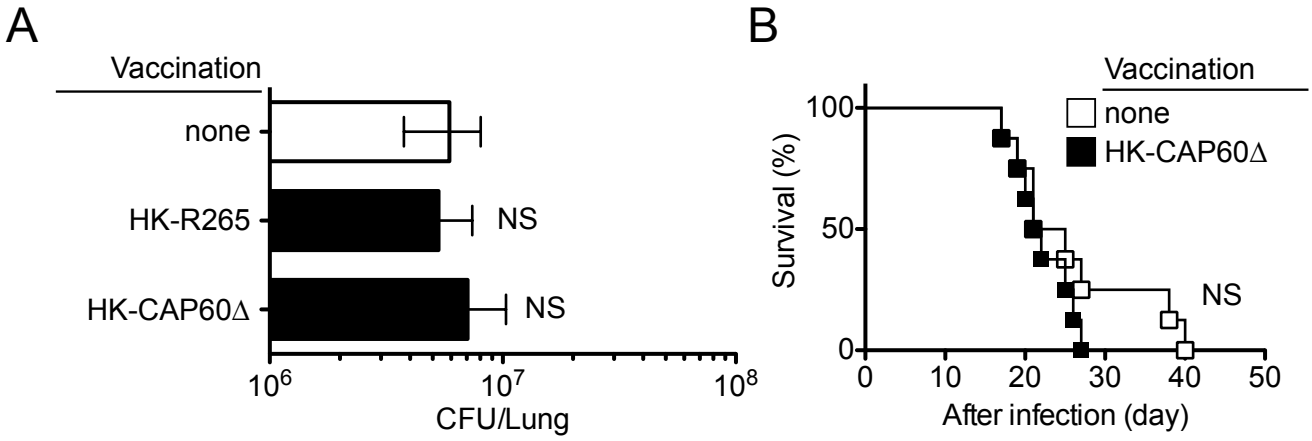

FIG S4 Vaccination using heat-killed fungal cells does not ameliorate pulmonary infection with highly virulent *C. gattii* strain R265. Heat-killed fungal cells, HK-R265 or HK-CAP60Δ (5×10^6 cells/mouse), were administered intravenously to mice, both at 14 days and at 1 day prior to intratracheal infection with R265 (3×10^3 CFUs/mouse). Fungal burdens at day 14 post-infection (A) and survival rates (B) were determined as described in Figure 2. Median survivals: non-vaccination = 23 days; HK-CAP60Δ transfer = 21.5 days.

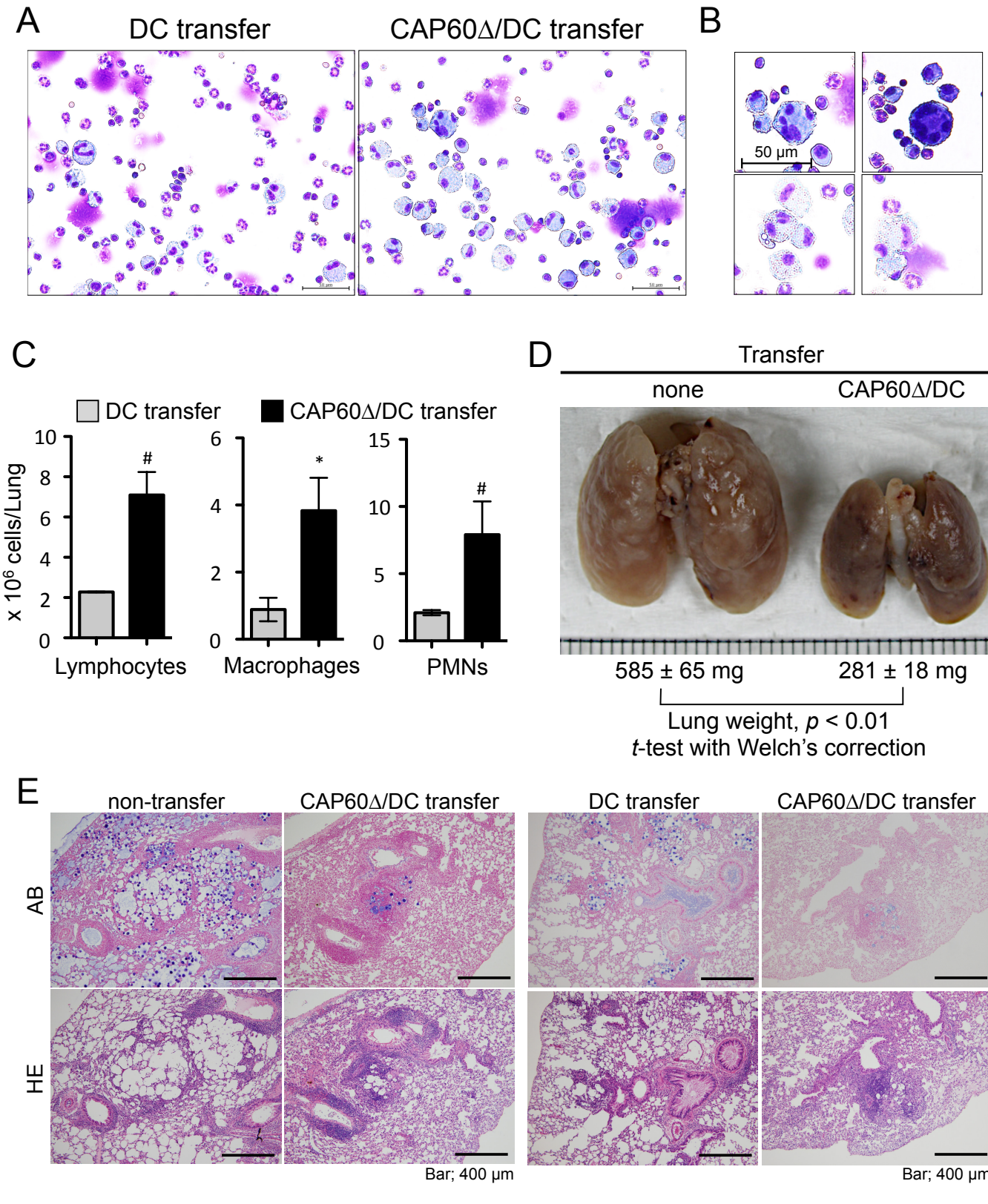

FIG S5 DC-based vaccine induces lung leukocyte recruitment and ameliorates lung pathology. Lung leukocytes were stained using Diff-Quick (A-B) and cell numbers were determined (C) at day 7 post-transfer. Multi-nucleated cells were found in the lungs of CAP60Δ/DC injected mice (B). Histograms show results expressed as means ± SD, * $p < 0.05$ vs. control by unpaired *t*-test, # $p < 0.05$ vs. control by unpaired *t*-test with Welch's correction. Similar results were obtained at day 9 post-transfer (C). Gross pathology and lung sections were assessed at day 13 post-infection (D-E). Lung sections were stained with hematoxylin-eosin [HE] or alcian blue [AB], as described in Figure 3, and observed at a magnification of ×100.

TABLE S2 Statistical analysis for lung sections

Three mice per group for evaluation	non-transfer	CAP60Δ/DC	*p*-value
A. Alveolar space (Cross point interval, μm)	68.6 ± 38.0 n = 1072	52.4 ± 25.7 n = 1038	$p < 0.01$
B. Variance of alveolar space (Variance of cross point interval, μm)	38.0 ± 0.753 n = 3	25.6 ± 1.06 n = 3	$p < 0.01$
C. The number of MGCs (MGCs/mm^2)	0.43 ± 0.14 n = 6	1.1 ± 0.46 n = 6	$p < 0.05$
D. The number of nuclei per MGC	3.7 ± 2.2 n = 273	7.0 ± 5.5 n = 378	$p < 0.01$
E. Nuclear density in MGC (nuclei/1000 μm^2)	4.2 ± 2.4 n = 116	8.8 ± 4.4 n = 108	$p < 0.01$

Detail methods are described in Material and Methods. Values are means ± SD. *P*-value in Column B was derived from unpaired *t*-test and the other *p*-values were derived from unpaired *t*-tests with Welch's correction.

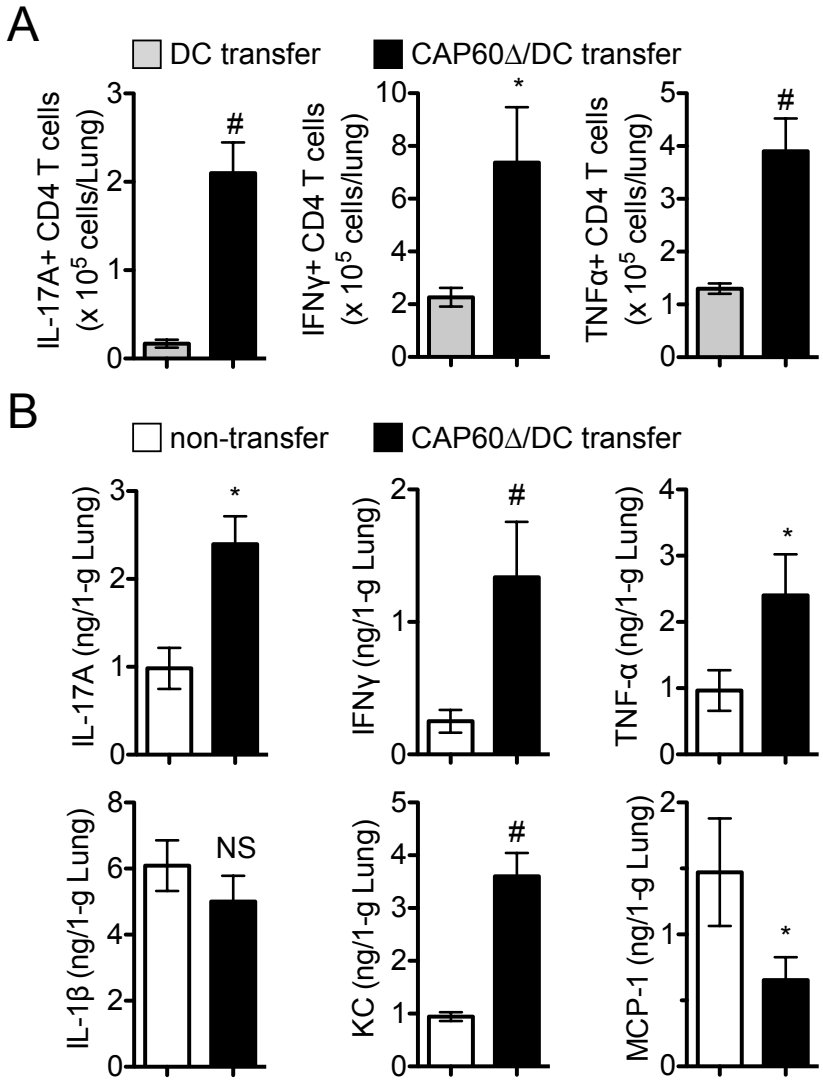

FIG S6 Transferring CAP60Δ/DCs induces cytokine production in lung. (A) Lung leukocytes were collected from three mice at 7 days post-transfer and stimulated with PMA and ionomycin for 3 h. For flow cytometry analysis, gates were set for CD3+ cells. Total numbers of CD4 T cells that expressed intracellular IL-17A, IFNγ, or TNFα were determined. Representative data from two separate experiments are expressed as means ± SD, * $p < 0.05$ vs. control by unpaired *t*-test, # $p < 0.05$ vs. control by unpaired *t*-test with Welch's correction. (B) Lungs were harvested from five mice at 14 days post-injection. Cytokine levels in lung homogenates were determined by ELISA. Representative data from three or four independent experiments are shown. Histogram results are means ± SD, * $p < 0.05$ vs. control by unpaired *t*-test, # $p < 0.05$ vs. control by unpaired *t*-test with Welch's correction.