Supplemental Figures Legend

Supplemental Figure 1

***p47$^{phox-/-}$* mice fail to develop vaccine-induced resistance to inactivated *A. fumigatus* conidia.** Mice (6/group) received inactivated *A. fumigatus* conidia intranasally 14 days before re-infection with *Aspergillus* conidia intranasally. Mice were given cyclophosphamide a day before re-infection. Resistance to re-infection was assessed in terms of (**A**) fungal growth (log_{10}CFU ± SEM), (**B**) lung histology (PAS staining) and (**C**) cytokine production (ELISA) by purified lung T cells cultured in vitro with conidia-pulsed dendritic cells from the corresponding mouse strain. In **B**, numbers refer to % polymorphonuclear (PMN) or mononuclear (MNC) cells in the BAL. Assays were done at 3 days post-infection. Data are pooled or representative (histology) from 2 independent experiments.*, P<0.05; **, P<0.01; ***, P<0.001, vaccinated *vs* unvaccinated (None) mice.

Supplemental Figure 2

Pep1p-coated microparticles induce robust CD8$^+$T cell activation. Mice (6/group) received 5µg Pep1p+50 µg microparticles, or microparticles alone, administered 14, 7 and 3 days before the infection with live *Aspergillus* conidia intranasally. FACS analysis showed that more than 90% of microparticles were fluorescent either immediately or 24 h after coupling with Alexa Fluor 488-conjugated Pep1p conjugation, thus showing a saturated and stable attachment of Pep1p to the microparticles. Resistance to re-infection was assessed in terms of (**A, B**) fungal growth (log_{10}CFU ± SEM), (**C**) lung histology (PAS staining) and (**D**) proliferation of CD4$^+$ or CD8$^+$T cells purified from lungs or Pep1p+microparticles-vaccinated mice and re-stimulated in vitro with Pep1p- or Pep1p+microparticles-pulsed naive DCs for 72 h. DNA synthesis was measured by ^{3}H-thymidine uptake (cpm, counts per minute).*, P<0.05; **, P<0.01; ***, P<0.001, vaccinated *vs* unvaccinated (None) or treated vs untreated (None) mice. Data are pooled or representative (histology) from 2 independent experiments. Concomitant administration of CPG did not modify the vaccinating potential of Pep1p+microparticles (data not shown).

Supplemental Figure 3

Failure of CpG alone to confer vaccine-induced resistance. *p47$^{phox-/-}$* mice were treated with CpG alone (**A, B**) or with CpG plus the fungal aspartic protease (Pep1p) (**C, D**) and assessed for resistance to re-infection 14 (**A** and **B**) or 60 (**C** and **D**) days later in terms of (**A, D**) fungal growth in the lung (log_{10}CFU ± SEM, assessed 3 days after re-infection); (**B**) lung histology (PAS staining and Gomori staining in the insets) and (**C**) survival (%). Mice were given cyclophosphamide a day before re-infection. Data are pooled from 2 independent experiments. ***, P<0.001, CpG-treated vs untreated (None) mice (**A**) and Pep1p+CpG-vaccinated *vs* unvaccinated (None) mice. Data are representative of two experiments.

Supplemental Figure 4

***p47$^{phox-/-}$* mice develop vaccine-induce resistance to recombinant fungal antigens.** *p47$^{phox-/-}$* mice were given the 1,3-beta glucanosyltransferase (Gel1p), the purified cell wall glucanase (Crf1p) and the metalloprotease (Mep1p) together with CpG 14, 7 and 3 days before re-infection with *Aspergillus* conidia intranasally. Three days later, mice were assessed for resistance to re-infection in terms of (**A**) survival (%); (**B**) fungal growth (log_{10}CFU ± SEM) and (**C**) lung histology (PAS staining and Gomori staining in the

insets). In **C**, numbers refer to % polymorphonuclear (PMN) or mononuclear (MNC) cells in the BAL at 3 days after re-infection. Data are pooled or representative (histology) from 3 independent experiments. ***, P<0.001, vaccinated *vs* unvaccinated (None) mice.

Supplemental Figure 5

Vaccine-induced resistance to *A. fumigatus* is mediated by Th1 and, partly, Th17 cells. *Il17ra*$^{-/-}$ or *Ifng*$^{-/-}$ mice were administered with *A. fumigatus* conidia or the protective recombinant fungal aspartic protease (Pep1p) with CpG as in legend to figure 1, and assessed for resistance to re-infection in terms of (**A**) survival (%); (**B**) fungal growth (log_{10}CFU ± SEM) and (**C**) pattern of cytokine gene expression by RT-PCR on RNA from total lung cells. (**D**) Conidiocidal activity [percentage of colony forming unit inhibition (mean ± SEM)] and (**E**) expression of mBD1 and cathelicidin mRNA by RT-PCR in vaccinated mice. RT-PCR and the conidiocidal assays were done on total lung cells. Data are pooled from 2 independent experiments. *, P<0.05; **, P<0.01; ***, P<0.001, vaccinated *vs* unvaccinated (None) mice and *p47*$^{phox-/-}$ *vs* C57BL6 mice (**D, E**).

Supplemental Figure 6

CD8$^{-/-}$ and CD4$^{-/-}$ mice fail to develop vaccine-induced resistance to *Aspergillus fumigatus* conidia or Pep1p antigen. Mice received *A. fumigatus* conidia 14 days before or Pep1p with CpG 14, 7 and 3 days before re-infection with *Aspergillus* conidia. Mice were assessed for resistance to re-infection in terms of (**A**) fungal growth (log_{10}CFU ± SEM) and (**B**) lung histology (PAS staining) 3 days after re-infection. Data are pooled or representative (histology) from 3 independent experiments. None, unvaccinated mice.

Supplemental Figure 7

***p47*$^{phox-/-}$ mice are not more susceptible than C57BL/6 mice to murine Cytomegalovirus infection.** Mice were infected intraperitoneally with 5x10^5 plaque-forming units (PFU) of the Smith strain MCMV and were assessed for: (**A**) virus titer, expressed as log_{10}PFU/gram of lung ± SEM, at 7 days after infection; (**B**) lung histology (Hematoxylin- and Eosin-staining); (**C**) frequency of IFN-□- and granzyme B(GrzB)-producing and (**D**) cytotoxic activity of CD8$^+$T cells. T-cell cytolytic activity was assessed by standard ^{51}Cr-release assay against dendritic cells using different Effector:Target (E:T) cell ratios. The frequency of IFN-□- or GrzB-producing cells was determined by ELISPOT assay on CD8$^+$T cells purified from spleens. Results are expressed as the number of cytokine-producing cells (mean ± SEM) per 10^5 cells, calculated using replicates of serial 2-fold dilutions of cells. Data are pooled or representative (histology) from 2 independent experiments. *, P<0.05; **, P<0.01; ***, P<0.001, infected *vs* uninfected mice.

Supplemental Figure 8

TLR3 expression and responsiveness in *p47*$^{phox-/-}$ mice. C57BL/6 or *p47*$^{phox-/-}$ mice were infected intranasally with live *A. fumigatus* conidia (6-8 mice/group). Relative expression of *Tlr3*, *Ifnb* and *Ifna* in the lungs of infected mice were assessed at 1 and 7 days post-infection by RT-PCR. 0, uninfected mice. Data are representative from 3 independent experiments.*, P<0.05; **, P<0.01; ***, P<0.001, *p47*$^{phox-/-}$ vs C57BL/6 mice.

Supplemental Figure 9

The intracellular routing of the *Aspergillus* metalloprotease Mep1p antigen in dendritic cells. Immunofluorescence imaging of purified dendritic cells from lungs of C7BL/6 mice after in vitro exposure to the Mep1p-Alexa Fluor 488 at 37°C for 2 h and chased for 15 and 45 min. Formaldehyde-fixed DCs were incubated with primary antibodies against Mannose Receptor (MR), Rab5, Rab7, Rab9, Rab14 and Lamp1 followed by secondary anti-rabbit IgG TRITC antibody. Nuclei were counterstained with DAPI. Images were acquired using a fluorescence microscope (BX51 Olympus) with a 100 × objective and the analySIS image processing software (Olympus). Shown are merging images of DCs (a single cell is magnified in the inset) pulsed with GFP-conidia or fungal antigens (green) and red-stained for each endosomal compartment. Shown are representative data from 2 independent experiments. Numbers refer to co-localization coefficients to quantify the degree of overlap.

Supplemental Figure 10

Flow cytometry of lung dendritic cells in C57BL6 or *p47*$^{phox-/-}$ mice. Numbers refer to % positive cells on T and B cell-depleted lung cells from uninfected mice. The data show that cross-presenting CD11b^{low}CD103^{+}DCs or CD8α^{+}DCs and tolerogenic B220^{+} or Siglec H^{+} DCs were not defective in the lungs of CGD mice. Data are representative of one experiment out of three.

Supplemental Figure 11

Flow cytometry of lung CD8^{+}T cells upon treatment with anti-CD8 or anti-MHC Class I antibody. Mice (6/group) received *A. fumigatus* conidia intranasally and concomitantly treated with 300 µg of anti-CD8, 34-5-8S (reacting with the H-2Dd MHC class I alloantigen and cross-reacting with cells from mice of the H-2Db haplotype) or 28-14-8 (reacting with the H-2Db MHC class I alloantigen). Untreated mice received isotype control mAb. The numbers refer to % positive cells by FACS analysis of total lung cells at different days post-infection. Data are representative of one experiment out of two.

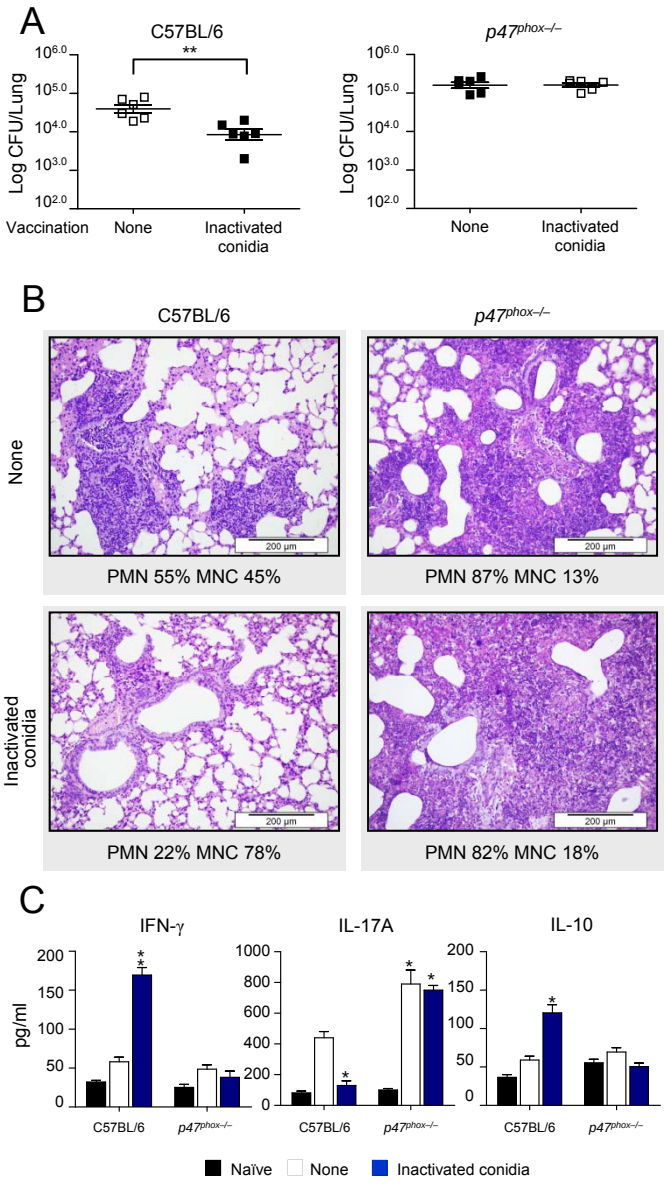
A
C57BL/6
p47phox−/−
**
Log CFU/Lung
10^6.0
10^5.0
10^4.0
10^3.0
10^2.0
Vaccination
None
Inactivated conidia
B
C57BL/6
p47phox−/−
None
Inactivated conidia
200 µm
PMN 55% MNC 45%
PMN 87% MNC 13%
PMN 22% MNC 78%
PMN 82% MNC 18%
C
IFN-γ
IL-17A
IL-10
pg/ml
200
150
100
50
0
1000
800
600
400
200
C57BL/6
p47phox−/−
Naïve
None
Inactivated conidia

Figure S1

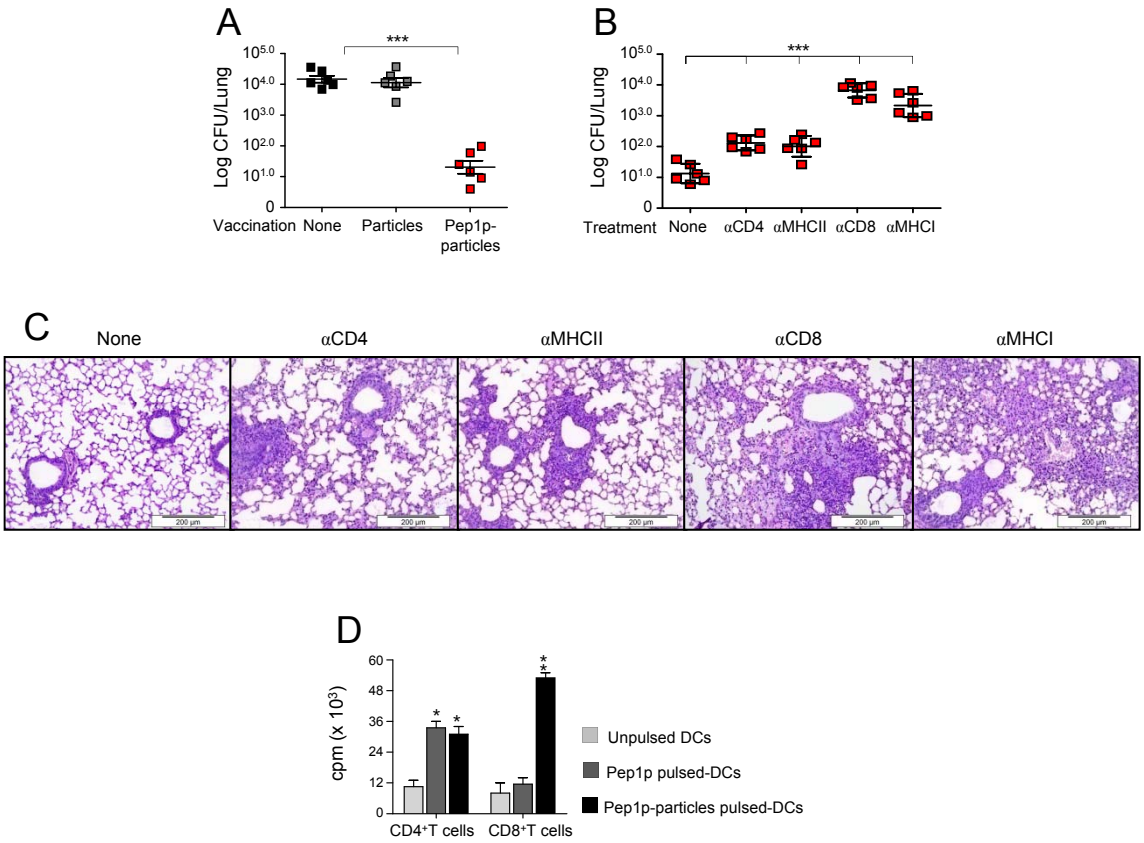
A
B
C
D
Log CFU/Lung
Vaccination None Particles Pep1p-particles
Treatment None αCD4 αMHCII αCD8 αMHCI
None
αCD4
αMHCII
αCD8
αMHCI
cpm (x 10³)
CD4⁺T cells
CD8⁺T cells
Unpulsed DCs
Pep1p pulsed-DCs
Pep1p-particles pulsed-DCs

Figure S2

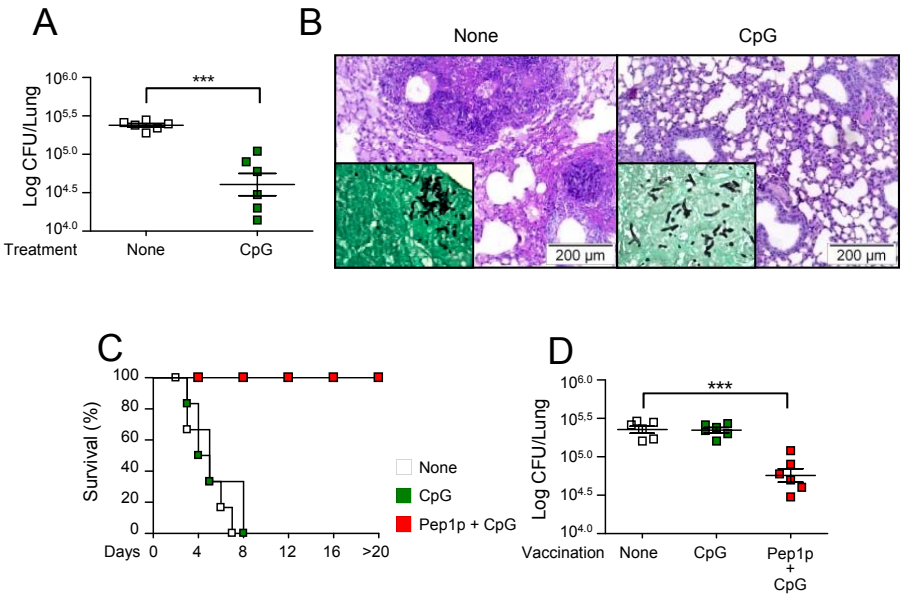
A
B
None
CpG
Log CFU/Lung
$10^{6.0}$
$10^{5.5}$
$10^{5.0}$
$10^{4.5}$
$10^{4.0}$
***
Treatment
None
CpG
200 µm
200 µm
C
Survival (%)
100
80
60
40
20
0
Days 0
4
8
12
16
>20
None
CpG
Pep1p + CpG
D
Log CFU/Lung
$10^{6.0}$
$10^{5.5}$
$10^{5.0}$
$10^{4.5}$
$10^{4.0}$
***
Vaccination
None
CpG
Pep1p
+
CpG

Figure S3

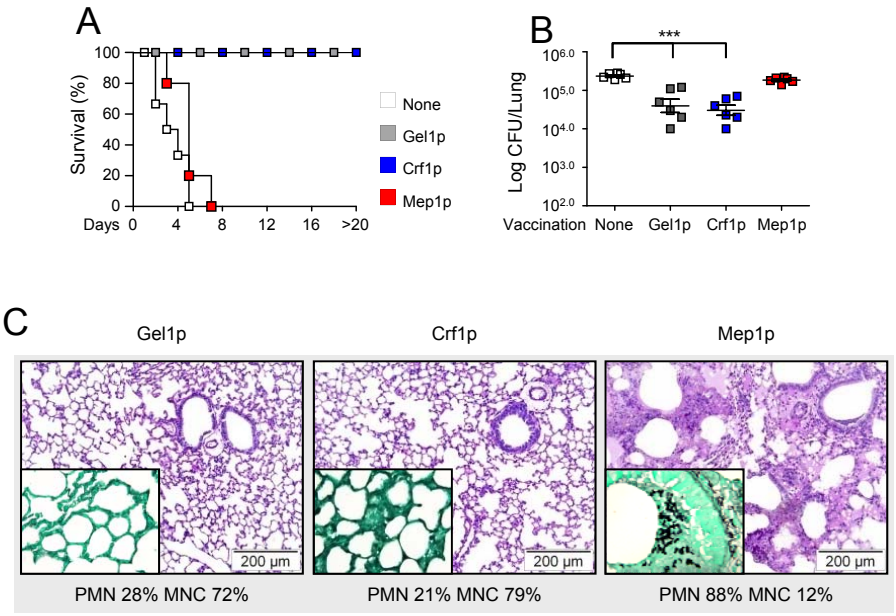
A
Survival (%)
100
80
60
40
20
0
Days 0 4 8 12 16 >20
None
Gel1p
Crf1p
Mep1p
B
***
Log CFU/Lung
$10^{6.0}$
$10^{5.0}$
$10^{4.0}$
$10^{3.0}$
$10^{2.0}$
Vaccination None Gel1p Crf1p Mep1p
C
Gel1p
Crf1p
Mep1p
200 µm
200 µm
200 µm
PMN 28% MNC 72%
PMN 21% MNC 79%
PMN 88% MNC 12%

Figure S4

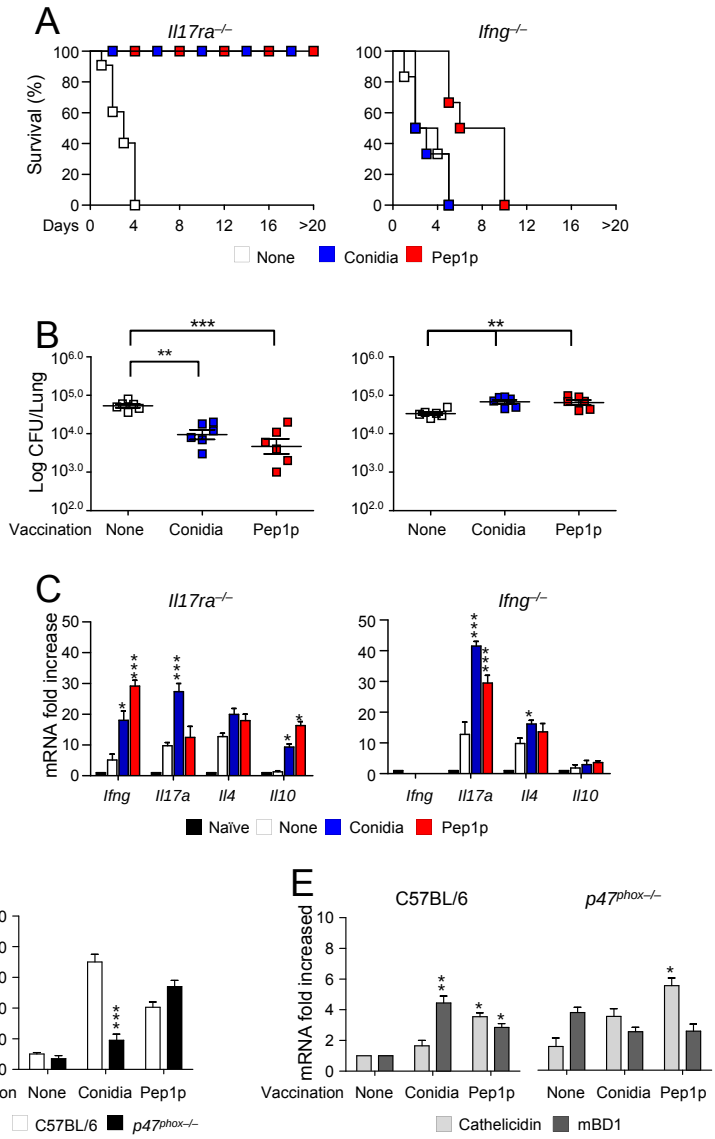
A
Il17ra−/−
Ifng−/−
Survival (%)
Days 0 4 8 12 16 >20
None Conidia Pep1p
B
Log CFU/Lung
Vaccination None Conidia Pep1p
C
Il17ra−/−
Ifng−/−
mRNA fold increase
Ifng Il17a Il4 Il10
Naïve None Conidia Pep1p
D
Conidiocidal activity (%)
Vaccination None Conidia Pep1p
C57BL/6 p47phox−/−
E
C57BL/6
p47phox−/−
mRNA fold increased
Vaccination None Conidia Pep1p
Cathelicidin mBD1

Figure S5

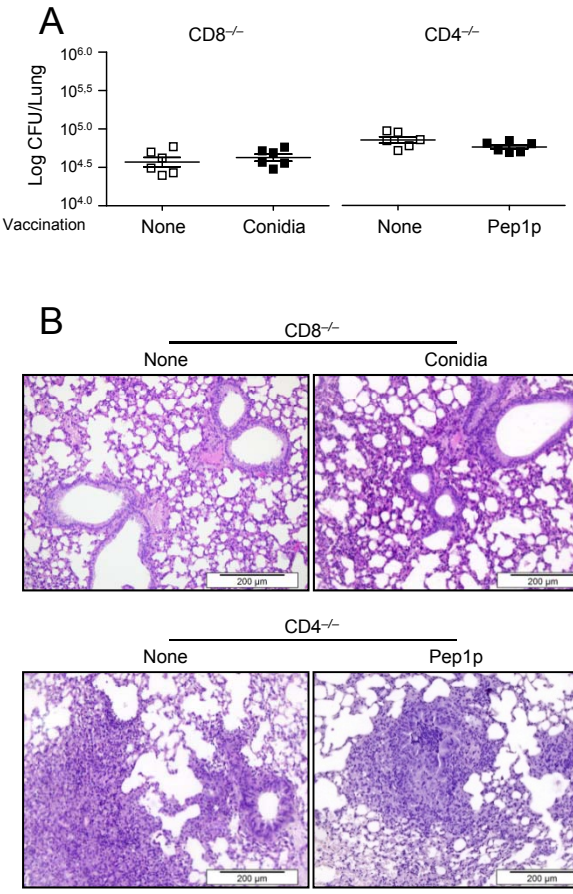
A
CD8−/−
CD4−/−
Log CFU/Lung
10^6.0
10^5.5
10^5.0
10^4.5
10^4.0
Vaccination
None
Conidia
None
Pep1p
B
CD8−/−
None
Conidia
200 µm
200 µm
CD4−/−
None
Pep1p
200 µm
200 µm

Figure S6

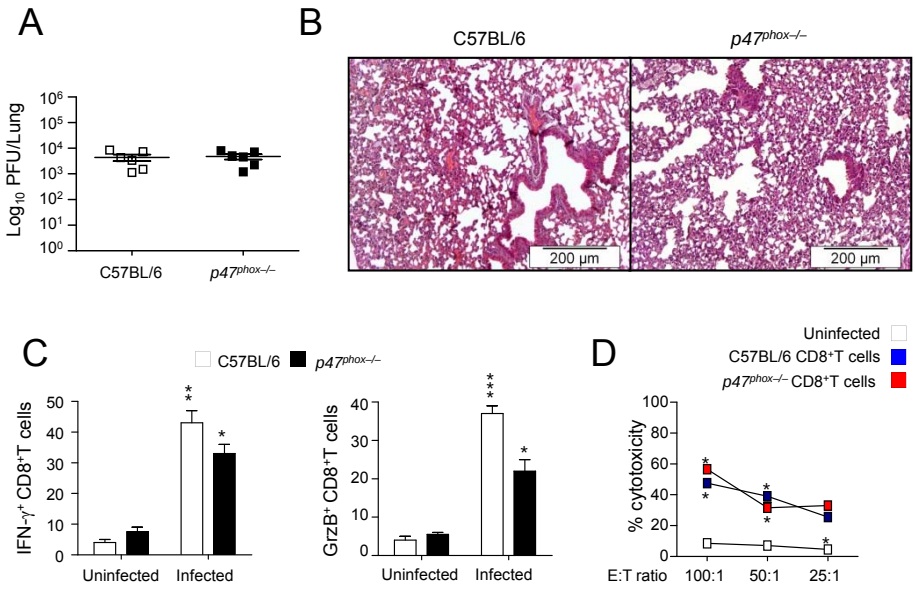
A
B
C
D
C57BL/6
p47phox–/–
Log10 PFU/Lung
200 µm
IFN-γ+ CD8+T cells
GrzB+ CD8+T cells
Uninfected
Infected
% cytotoxicity
E:T ratio
100:1
50:1
25:1
C57BL/6 CD8+T cells
p47phox–/– CD8+T cells

Figure S7

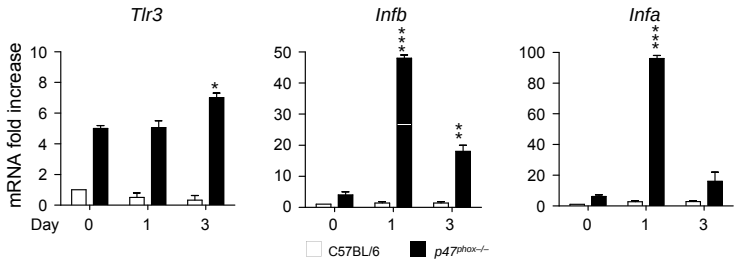

Figure S8

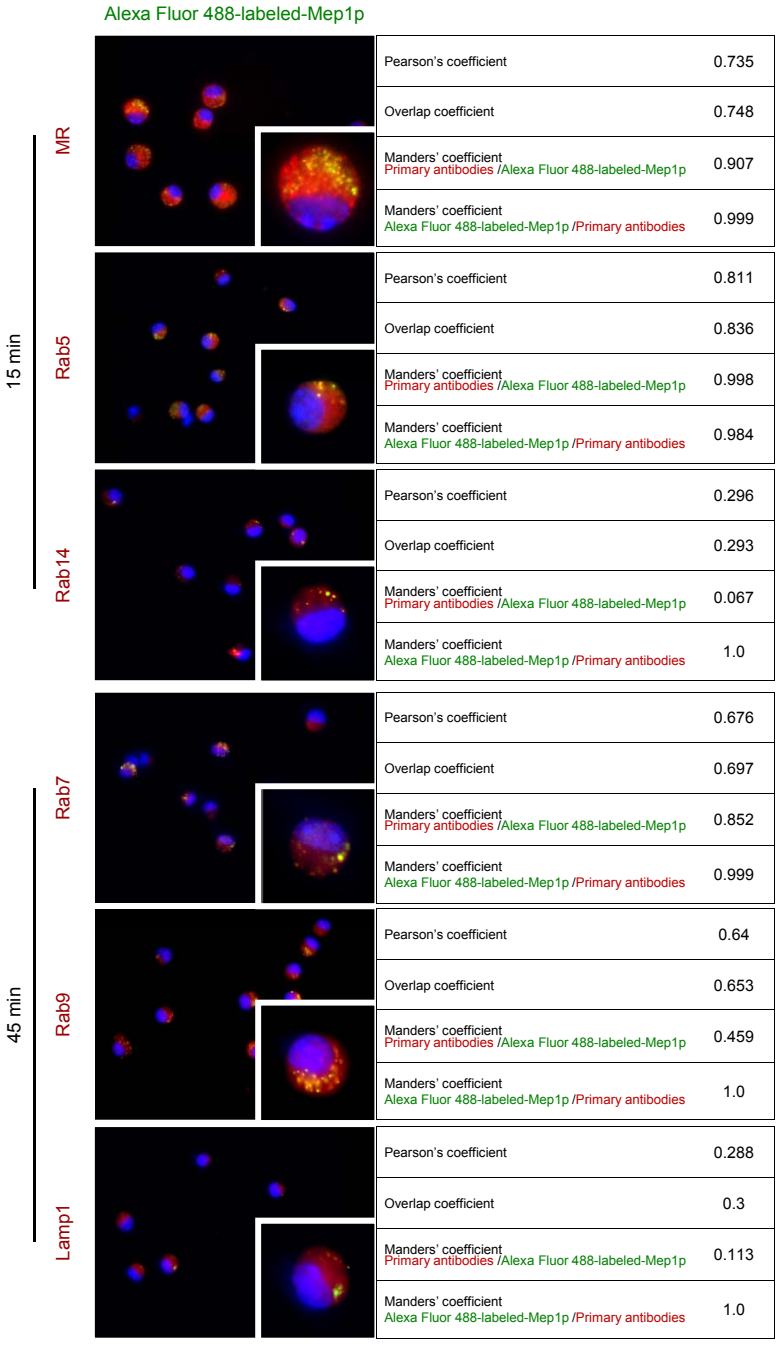
Alexa Fluor 488-labeled-Mep1p
15 min
45 min
MR
Rab5
Rab14
Rab7
Rab9
Lamp1
Pearson's coefficient 0.735
Overlap coefficient 0.748
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.907
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 0.999
Pearson's coefficient 0.811
Overlap coefficient 0.836
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.998
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 0.984
Pearson's coefficient 0.296
Overlap coefficient 0.293
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.067
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 1.0
Pearson's coefficient 0.676
Overlap coefficient 0.697
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.852
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 0.999
Pearson's coefficient 0.64
Overlap coefficient 0.653
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.459
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 1.0
Pearson's coefficient 0.288
Overlap coefficient 0.3
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Mep1p 0.113
Manders' coefficient Alexa Fluor 488-labeled-Mep1p /Primary antibodies 1.0

Figure S9

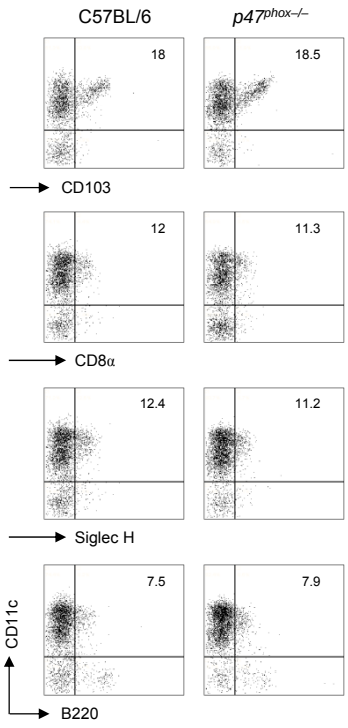
C57BL/6
p47phox–/–
18
18.5
CD103
12
11.3
CD8α
12.4
11.2
Siglec H
7.5
7.9
CD11c
B220

Figure S10

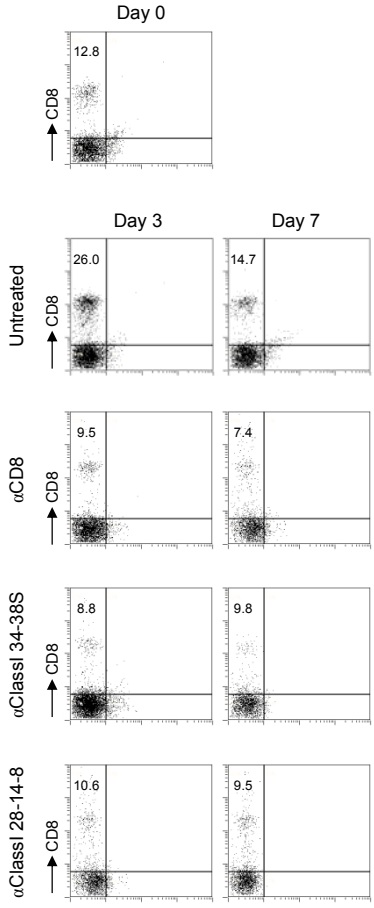
Day 0
12.8
CD8
Day 3
Day 7
Untreated
26.0
14.7
αCD8
9.5
7.4
αClassI 34-38S
8.8
9.8
αClassI 28-14-8
10.6
9.5

Figure S11

Supplemental Table 1. Co-localization coefficients

	15 min			45 min			Rab14	
	MR	**Rab5**	**Rab14**	**Rab7**	**Rab9**	**Lamp1**	3-MA	Chloroquine
C57BL/6 GFP - *Aspergillus* conidia								
Pearson's coefficient	0.551	0.304	0.275	0.325	0.491	0.638	0.055	0.366
Overlap coefficient	0.671	0.407	0.354	0.385	0.542	0.758	0.059	0.392
Manders' coefficient Primary antibodies /GFP - *Aspergillus* conidia	0.622	0.304	0.22	0.327	0.579	0.884	0.004	0.187
Manders' coefficient GFP - *Aspergillus* conidia/Primary antibodies	0.976	0.999	0.967	1.0	0.99	1.0	1.0	1.0
Alexa Fluor 488-labeled-Pep1p								
Pearson's coefficient	0.75	0.68	0.134	0.309	0.245	0.802	0.114	0.193
Overlap coefficient	0.771	0.686	0.141	0.318	0.245	0.85	0.11	0.189
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Pep1p	0.805	0.597	0.13	0.12	0.051	0.942	0.005	0.029
Manders' coefficient Alexa Fluor 488-labeled-Pep1p /Primary antibodies	1.0	1.0	1.0	1.0	1.0	0.991	1.0	1.0
p47$^{phox-/-}$ **GFP - *Aspergillus* conidia**								
Pearson's coefficient	0.332	0.426	0.012	0.48	0.263	0.545	0.057	0.095
Overlap coefficient	0.478	0.588	0.014	0.555	0.466	0.695	0.056	0.104
Manders' coefficient Primary antibodies /GFP - *Aspergillus* conidia	0.504	0.705	0.0	0.463	0.349	0.989	0.002	0.017
Manders' coefficient GFP - *Aspergillus* conidia/Primary antibodies	1.0	0.999	1.0	1.0	0.999	0.98	1.0	1.0
Alexa Fluor 488-labeled-Pep1p								
Pearson's coefficient	0.823	0.595	0.253	0.547	0.591	0.786	0.184	0.556
Overlap coefficient	0.872	0.603	0.249	0.553	0.611	0.808	0.205	0.562
Manders' coefficient Primary antibodies /Alexa Fluor 488-labeled-Pep1p	0.997	0.376	0.057	0.269	0.649	0.742	0.076	0.27
Manders' coefficient Alexa Fluor 488-labeled-Pep1p /Primary antibodies	0.916	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The co-localization program Fiji with the JACoP Plugin was used to quantify the degree of overlap by calculating the co-localization coefficients (Pearson's correlation coefficient, Overlap coefficient according to Manders and the Overlap coefficients). The numbers in red denote the most important differences.