Figure S1. p40$^{phox+/-}$ neutrophils display normal levels of ROS production.

(A) Lysates from 4x10 ^{5} wt (+/+), p40$^{phox+/-}$(+/-) or p40$^{phox-/-}$(-/-) BMN were subjected to SDS-PAGE and immunoblotted for p40^{phox}, p67^{phox} and p47^{phox} as described in Materials and Methods. A representative experiment of three is shown. (B) 1x10^{6} wt, p40$^{phox+/-}$ or p40$^{phox-/-}$ BMN were pre-incubated with luminol/HRP prior to addition to fMLP or PMA, or pre-incubated with luminol prior to addition to serum-*S.aureus* or IgG-SRBC, as described in Materials and Methods. Histograms represent total integrated ROS responses as a percentage of wt. All data are means ± SEM (n=3; performed in duplicate).

Figure S2. Acidic substitution of T154 does not increase activity of the NADPH oxidase.

(A) p40$^{phox-/-}$BMN reconstituted with ev, wt p40^{phox} or p40^{phox}-T154E were sonicated, subjected to SDS-PAGE and immunoblotted for *phox* components as described in Materials and Methods. A representative blot of three is shown. (B) Protein expression was quantified with Aida software, as described in Materials and Methods. Levels of p40^{phox} and p67^{phox} were normalised against levels of p47^{phox}, which was used as a loading control. Histograms represent relative levels of p40^{phox} (left) or p67^{phox} (right) as a percentage of wt. Data are means ± SEM (n=3 independent experiments performed in duplicate). (C-F) ROS production was measured in p40$^{phox-/-}$BMN reconstituted as indicated, as described in Figure 3B-E. Histograms represent total integrated ROS responses as a percentage of wt. All data are means ± SEM (n=3; performed in duplicate). Where indicated, differences between means of

wt and mutated p40^{phox} are statistically significant. *p=0.02; **p=0.004; as determined by a paired Student's *t*-test.

Figure S3. Structure of the SH3 domain of p40^{phox} in complex with the PP motif of p47^{phox}

Left: wt p40^{phox}. The position of W207 in the SH3 domain of p40^{phox} is indicated, as well as the position of R368 (blue) in the PP motif of p47^{phox}. Right: mutated p40^{phox} containing the W207Y substitution, which introduced an acidic hydroxyl group (indicated by the green arrow). Figure created with Deep View software. PDB code: 1W70.

Figure S4. T154A substitution on a p40^{phox}-ΔSH3 background does not increase activity of the NADPH oxidase.

(A) p40$^{phox-/-}$ BMN reconstituted with ev, wt p40^{phox} or p40^{phox}-ΔSH3/T154A were sonicated, subjected to SDS-PAGE and immunoblotted for *phox* components as described in Materials and Methods. A representative blot of three is shown. (B) Protein expression was quantified with Aida software, as described in Materials and Methods. Levels of p40^{phox} and p67^{phox} were normalised against levels of p47^{phox}, which was used as a loading control. Histograms represent relative levels of p40^{phox} (left) or p67^{phox} (right) as a percentage of wt. Data are means ± SEM (n=3 independent experiments performed in duplicate). (C-F) ROS production was measured in p40$^{phox-/-}$ BMN reconstituted as indicated, as described in Figure 3B-E. Histograms represent total integrated ROS responses as a percentage of wt. All data are means ± SEM (n=3; performed in duplicate). Where indicated, differences between means of

wt and mutated p40^{phox} are statistically significant. *p=0.002; **p=0.001; ***p=0.000; as determined by a paired Student's *t*-test.

Figure S5. Effect of PKC inhibitors, genetic ablation of PKCδ or wortmannin on ROS production.

(A) 1x10^6 BMN from wt C57BL/6J mice were pre-treated for 10 minutes with vehicle control (0.1% DMSO), BIM-1, Gö 6976 or Gö 6983 at a concentration of 1 μM, or with wortmannin at a concentration of 100 nM or 300 nM. Cells were then used in chemiluminescence assays for ROS production as described in Figure S1B. Histograms represent total integrated ROS responses as a percentage of vehicle control. All data are means ± SEM (n=3; performed in duplicate), with the exception of *S.aureus* + wortmannin or IgG-SRBC + wortmannin, where n=1 due to previous published and unpublished observations ([1] and S. Kulkarni, personal communication). (B) 1x10^6 wt or PKCδ$^{-/-}$ BMN were used in chemiluminescence assays for ROS production as described in Figure S1B. Histograms represent total integrated ROS responses as a percentage of wt. Data are means ± SEM (n=3; performed in duplicate).

Figure S6. p40$^{phox-/-}$ neutrophils display a large defect in translocation of p47^{phox} to *S.aureus* phagosomes.

5x10^4 wt or p40$^{phox-/-}$ BMN were incubated without or with 1x10^6 serum-opsonised *S.aureus* for 7 min at 37°C. Samples were processed and visualised as described in Figure 7. Shown are representative fluorescence and DIC images for conditions tested. Arrows indicate the position of internalised bacteria. Cytosolic and phagosomal accumulation of *phox* components was quantified for at least 50 phagocytic events under each condition using LSM

510 Image browser software. Data are presented as increase in fluorescence intensity over cytosolic levels (% of wt; mean ± SEM). **Difference between means of wt and $p40^{phox-/-}$ neutrophils is statistically significant. $p<0.001$ as determined by a paired Student's *t*-test.

REFERENCES

1. Ellson C, Davidson K, Anderson K, Stephens LR, Hawkins PT. PtdIns3P binding to the PX domain of p40phox is a physiological signal in NADPH oxidase activation. Embo J. 2006;25(19):4468-4478.

Figure S1

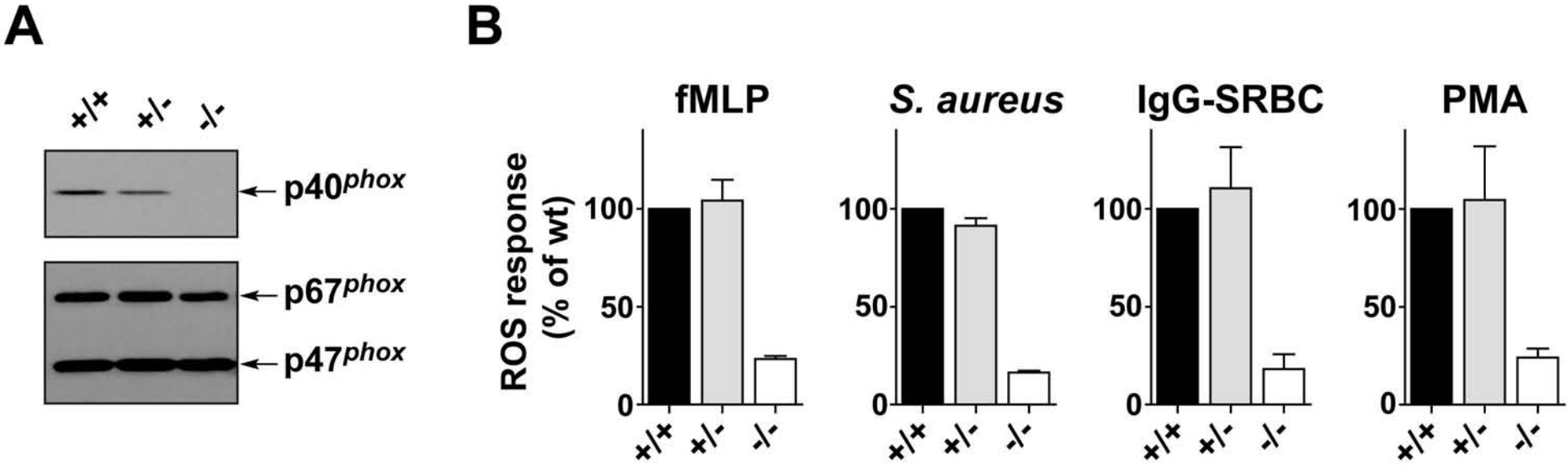

A
+/+
+/-
-/-
p40phox
p67phox
p47phox
B
fMLP
S. aureus
IgG-SRBC
PMA
ROS response
(% of wt)
100
50
0
+/+
+/-
-/-

Figure S2

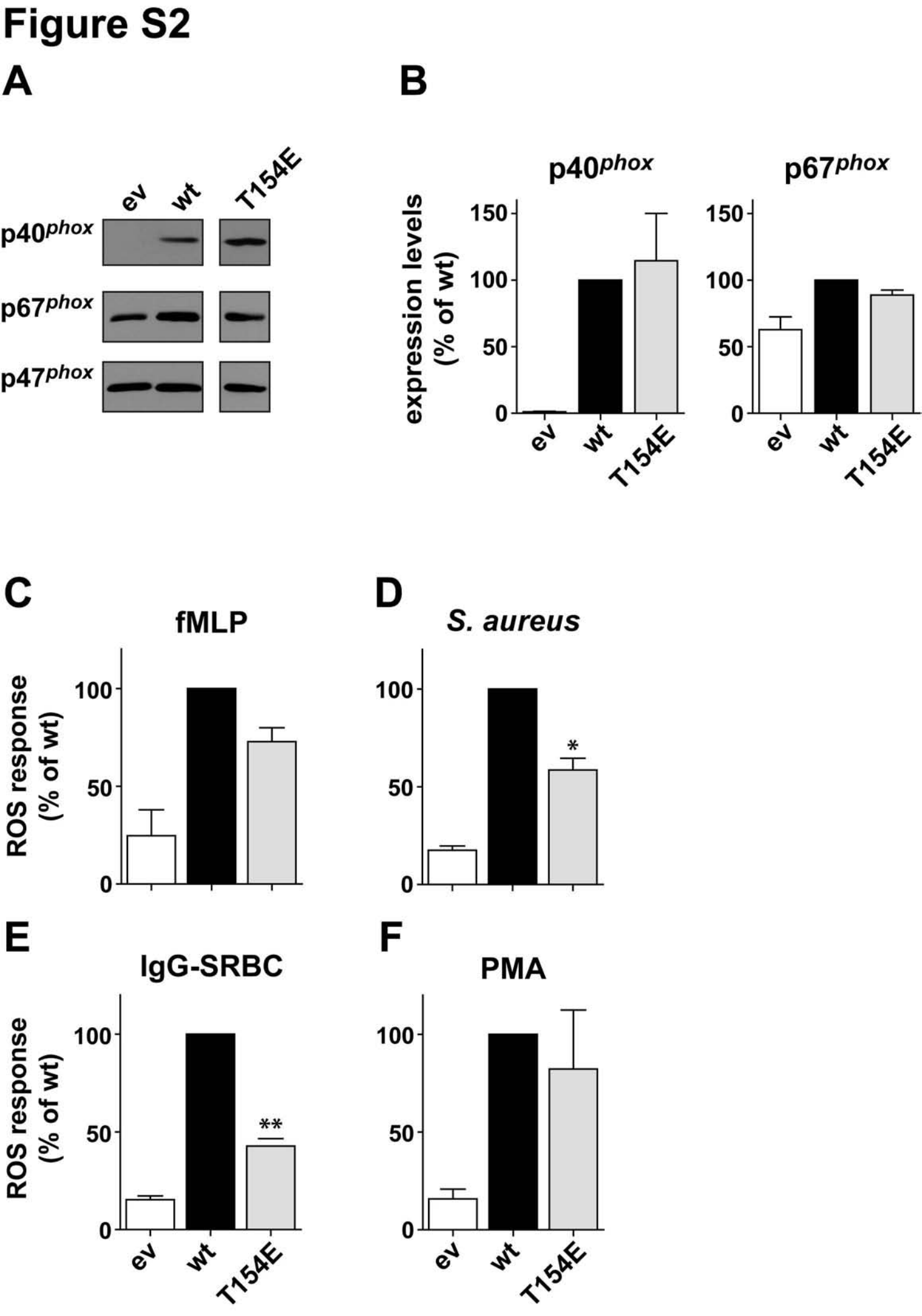
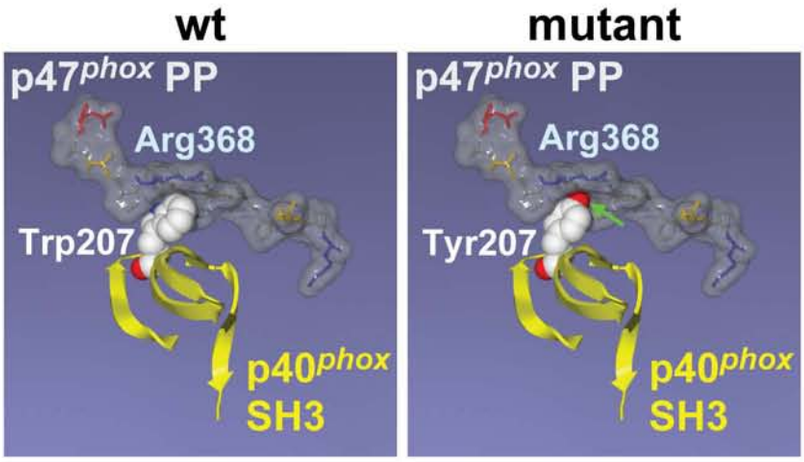

A
ev
wt
T154E
p40phox
p67phox
p47phox
B
p40phox
p67phox
expression levels (% of wt)
150
100
50
0
ev
wt
T154E
C
fMLP
ROS response (% of wt)
100
50
0
D
S. aureus
*
E
IgG-SRBC
**
F
PMA

Figure S3


wt
mutant
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Trp207
p40phox
SH3
p47phox PP
Arg368
Tyr207
p40phox
SH3

Figure S4

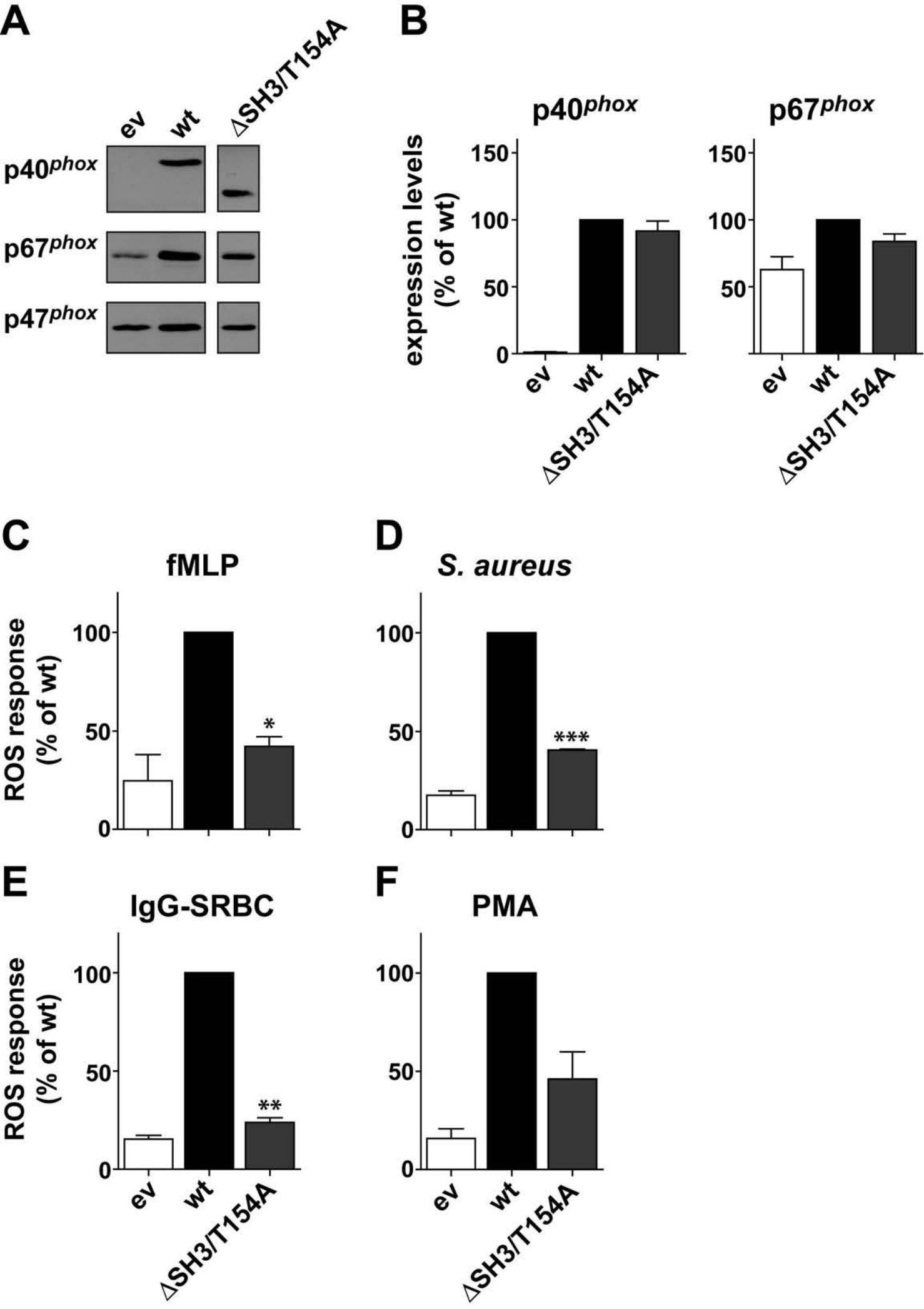

A
ev
wt
ΔSH3/T154A
p40phox
p67phox
p47phox
B
p40phox
p67phox
expression levels (% of wt)
150
100
50
0
ev
wt
ΔSH3/T154A
C
fMLP
ROS response (% of wt)
*
D
S. aureus
***
E
IgG-SRBC
**
F
PMA

Figure S5

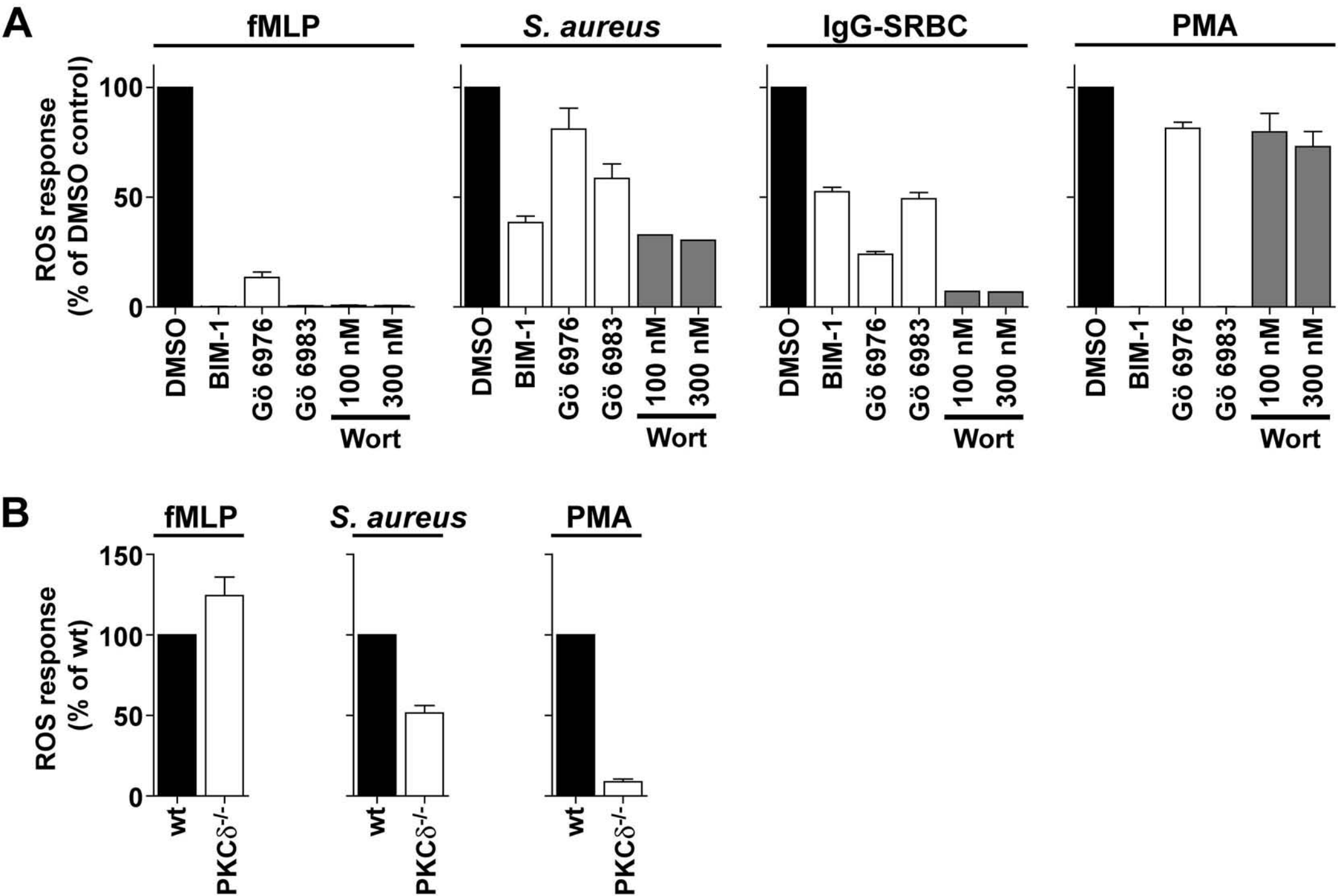
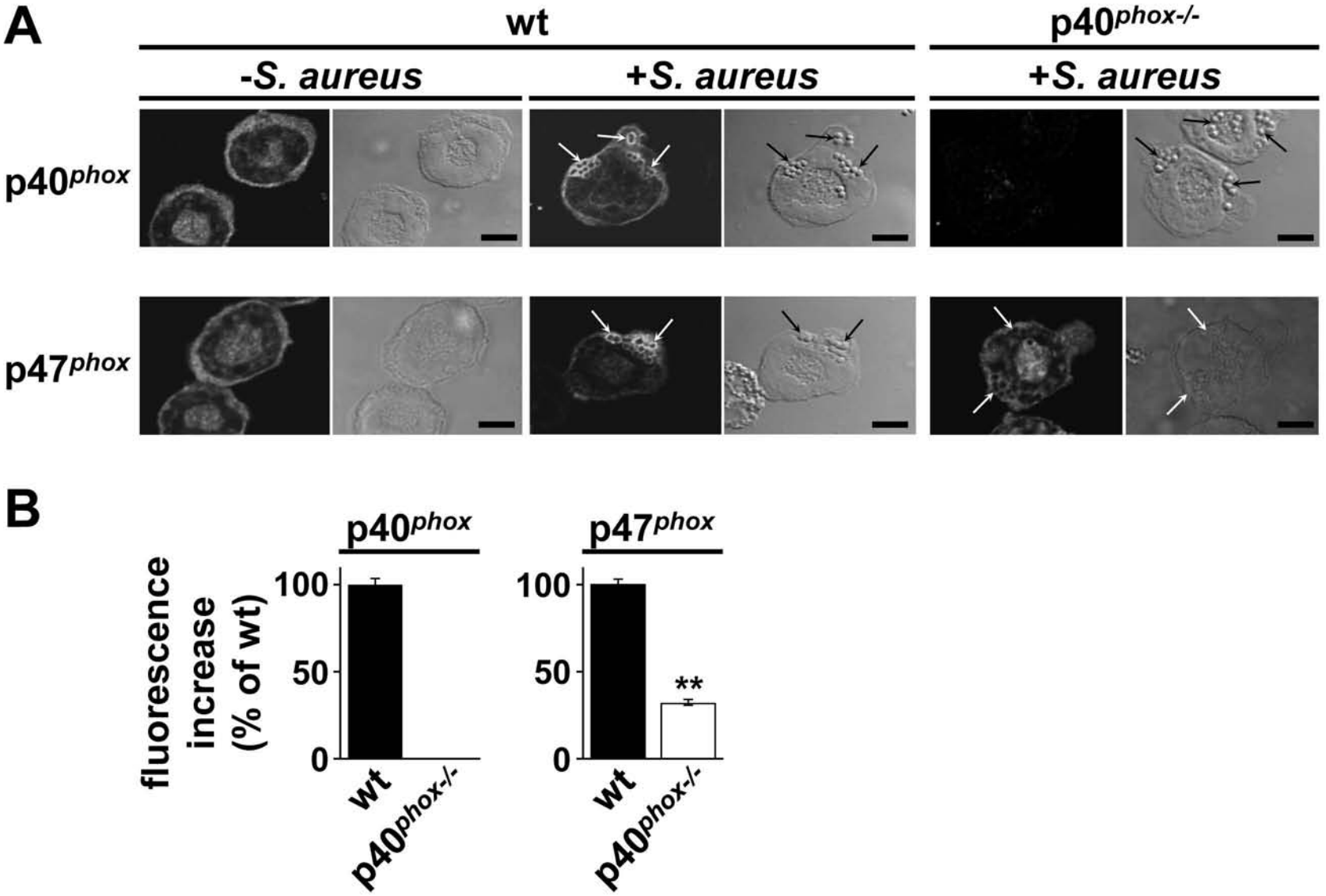

A
fMLP
S. aureus
IgG-SRBC
PMA
ROS response
(% of DMSO control)
100
50
0
DMSO
BIM-1
Gö 6976
Gö 6983
100 nM
300 nM
Wort
B
fMLP
S. aureus
PMA
ROS response
(% of wt)
150
100
50
0
wt
PKCδ-/-

Figure S6


A
wt
p40phox-/-
-S. aureus
+S. aureus
+S. aureus
p40phox
p47phox
B
p40phox
p47phox
fluorescence increase (% of wt)
100
50
0
**
wt
p40phox-/-