SUPPLEMENTAL INFORMATION TITLES AND LEGENDS

Pauline Basso[1*], Eric V. Dang[3], Anatoly Urisman[4], Leah E. Cowen[5], Hiten D. Madhani[3], Suzanne M. Noble[1,2 *#]

Figure S1. *lip2* undergoes normal morphogenesis under *in vitro* liquid hypha-inducing conditions, Related to Figure 1.

(A) *lip2* undergoes normal morphogenesis under *in vitro* liquid hypha-inducing conditions. WT *C. albicans* and *lip2* were incubated in YPD+5% serum, Spider, or RPMI for three hours at 30ºC or 37ºC, 5% CO_2.

(B) WT and *lip2* exhibit similar rates of germination. WT and *lip2* were incubated in liquid YPD+5% serum for 180 minutes, and samples were monitored at the indicated time points.

(C) *LIP2* is upregulated under hypha-inducing conditions. WT *C. albicans* was propagated in yeast-promoting (liquid YEPD) or hypha-inducing (liquid YEPD+5% serum or RPMI) medium at 30°C or 37°C to mid log growth (OD_{600}=1), and *LIP2* mRNA was quantified by RT-qPCR (and normalized to *ACT1*). Statistical significance between YEPD and the different media was determined by an unpaired two-tailed t-test; * p<0.05; **** p<0.0001.

Figure S2. Lip2 is required for kidney damage, Related to Figure 1.

(A) *lip2* exhibits a relative persistence defect in livers but not in spleens. Groups of BALB/c mice were infected with $1x10^5$ CFU of WT, *lip2,* or *lip2+LIP2,* followed by euthanasia of three animals per group at the indicated time points (note that the CFUs were assessed in the same animals evaluated for kidneys CFUs in Fig.1D). Statistical significance between WT and *lip2* determined by an unpaired two-tailed t-test. ns: p> 0.05.

(B) *lip2* forms microabscesses in kidneys at early time points but is subsequently cleared. 400X images of PAS-stained left kidneys from experiment described in Fig.1D and presented in Fig.1E.

(C) *lip2* exhibits gross defects in kidney invasion as early as 16 hours after infection. 40X images of PAS-stained left kidneys from experiment described in Fig.1D.

(D) *lip2* causes minimal gross kidney damage. 40X images of H&E-stained of left kidneys from experiment described in Fig.1D

Figure S3. *lip2* induce a strong Il-17 response in C57BL/6J mice, Related to Figure 2.

(A) *lip2* induces a strong renal IL-17A response during systemic infection. Groups of C57BL/6J mice were infected with $1x10^6$ CFU WT or *lip2,* followed by euthanasia of three animals per group at the indicated time points. *Il17a* mRNA was measured by RT-qPCR in the right kidney homogenates and normalized to *Gadph* (upper panel). IL-17A protein production was evaluated by ELISA in the left kidney homogenates (lower panel). Statistical significance between WT and *lip2* was determined by an unpaired two-tailed t-test. ** p< 0.01; ***p < 0.001; ****p< 0.0001.

(B) Genes for cytokines are expressed at similar levels in *Il17af*$^{-/-}$ C57BL/6J mice infected with *lip2* or WT *C. albicans*. Groups of *Il17af*$^{-/-}$ C57BL/6J mice were infected with $5x10^5$ CFU of WT or *lip2* followed by euthanasia of three animals per group at the indicated time points. *Il1b*, *Il6*, *Il10*, and *Ifng* mRNAs in kidney homogenates at the indicated time points were quantified by RT-qPCR (expression relative to *Gadph*). Statistical significance between WT and *lip2* was determined by an unpaired two-tailed t-test. * p< 0.05. (Note that points without visible error bars displayed SEMs smaller than the circle.)

(C) Representative flow cytometry plots and quantification of renal TCRγδ+ T cells producing IL-17A upon *lip2* stimulation (gated on CD45+ cells). Groups of C57BL/6J mice were infected with $1x10^6$ CFU of WT or *lip2* strains. Animals were euthanized after six hours of infection. Flow cytometry analysis was performed on total renal cells after four hours of stimulation with PMA, ionomycin and Golgi STOP (Note: see Fig. 3H for quantification). Statistical significance

between the different conditons was determined by one-way ANOVA (Tukey's multiple comparisons test); ns: p>0.05.

(D) Representative flow cytometry and quantification of renal TCR**γ**δ+ T cells producing IL-17A upon *lip2* stimulation. Groups of C57BL/6J SMART17 mice were infected with $1x10^6$ CFU of WT or *lip2*. Animals were euthanized after six hours of infection. Flow cytometry analysis was performed on total renal cells (gated on CD45+ cells). Statistical significance between the different conditions was determined by one-way ANOVA (Tukey's multiple comparisons test); ns: p>0.05; * p<0.05.

(C) Representative flow cytometry plots of IL-17A-producing CD4+, ILC3, TCR**γ**δ+ T cells in *lip2* infected kidneys. Groups of C57BL/6J mice were infected with normal saline ("mock") or $1x10^6$ CFU of WT or *lip2* and euthanized after six hours. Flow cytometry analysis was performed on total renal cells after four hours of stimulation with PMA, ionomycin and Golgi STOP. After back-gating on cells producing IL-17A, the cell types were defined as: CD4+ (CD45+, CD90+, GL3-, TCRβ+, CD4+), ILC3 (CD45+, CD90+, GL3-, TCRβ-, CD4-), TCR**γ**δ+ (CD45+, CD90+, GL3+, TCRβ-).

(D) Quantification of IL-17-producing ILC3 and CD4+ T cells in mock-infected, WT-infected, and *lip2*-infected kidneys. Plot reflects the values obtained from all animals described in S1C. Statistical significance of differences between the groups was determined by one-way ANOVA (Tukey's multiple comparisons test); ns p>0.05.

(E) Representative flow cytometry plots and quantitation of IL-17A-producing TCR**γ**δ+ T cells in *lip2*-infected kidneys. Groups of C57BL/6J SMART17 mice were infected with normal saline ("mock") or $1x10^6$ CFU of WT or *lip2* and euthanized after six hours. Flow cytometry analysis was performed on total renal cells (gated on CD45+ cells). Statistical significance between the different conditions was determined by one-way ANOVA (Tukey's multiple comparisons test); ns p>0.05; * p<0.05.

Figure S4. Analysis of renal cells during systemic candidaiasis, Related to Figure 4.

(A-B) Assessment of renal dendritic cells from C57B/6J mice during systemic candidiasis. Groups of C57BL/6J mice were infected with normal saline ("mock") or $1x10^6$ CFU of WT or *lip2* and euthanized after 6, 24 or 48 hours. Flow cytometry was performed on total renal cells. Cell types were defined as: macrophages (CD45+, B220-, CD11b and F480+), DCs (CD45+ CD11b+ CD11c+ MHCII+).

A) Representative flow cytometry plots of macrophages and dendritic cells in *lip2*-infected kidneys.

B) Quantification of renal DCs in mock-, WT-, and *lip2*-infected kidneys. Plots represent the number of DCs either as a % of CD45+ cells or on an absolute scale. Statistical significance of differences between WT and *lip2* was determined by an unpaired two-tailed t-test. ns $p>0.05$. (Note that data for macrophages was of insufficient quality for quantitation.)

(C-D) Assessment of renal dendritic cells from BALB/c mice during systemic candidiasis. Groups of BALB/c mice were infected with normal saline ("mock") or $1x10^5$ CFU of WT or *lip2* and euthanized after 6, 24 or 48 hours. Analysis was performed on total renal cells as in (A-B).

C) Representative flow cytometry plots of macrophages and dendritic cells in *lip2*-infected kidneys.

D) Quantification of renal macrophages in mock-, WT-, and *lip2*-infected kidneys. Plots represent the number of macrophages either as a % of CD45+ cells or on an absolute scale. Statistical significance between WT and *lip2* was determined by an unpaired two-tailed t-test. ns $p>0.05$.

(E) Gene expression of *LIP2, ECE1, EFG1, SAP6,* and *LIP10* by WT *C. albicans* under BMDC coculture conditions. WT was propagated in RPMI medium at 37°C in 5% CO2 for 2 hours, and mRNA levels were assessed by RT-qPCR, using *ACT1* as a normalization control.

Figure S5. Palmitic acid dampened the activation of renal dendritic cells, Related to Figure 6.

(A) Palmitic acid suppresses the activation of BMDCs by *lip2*. BMDCs from C57BL/6J mice were incubated with 0.01 μM, 0.1 μM or 1 μM of fatty acids (palmitic acid, stearic acid or linoleic acid) or chloroform (solvent) alone and co-cultured with WT or *lip2* at a MOI of 1 for two hours, followed by measurement of IL-23A in cell supernatants by ELISA. Statistical significance of differences between WT and *lip2* samples was determined by one-way ANOVA (Tukey's multiple comparisons test); ns: $p > 0.05$; *** $p < 0.001$.

(B) IL-23A is similarly expressed in RPMI in the absence or presence of chloroform. BMDCs from C57BL/6J mice were incubated with chloroform (solvent) and co-cultured with WT, *lip2* strain at a MOI of 1 for two hours followed by measurement of IL-23A in cell supernatants by ELISA. Statistical significance of the difference between the *lip2* samples under solvent and RPMI conditions was determined by one-way ANOVA (Tukey's multiple comparisons test); ns: $p > 0.05$.

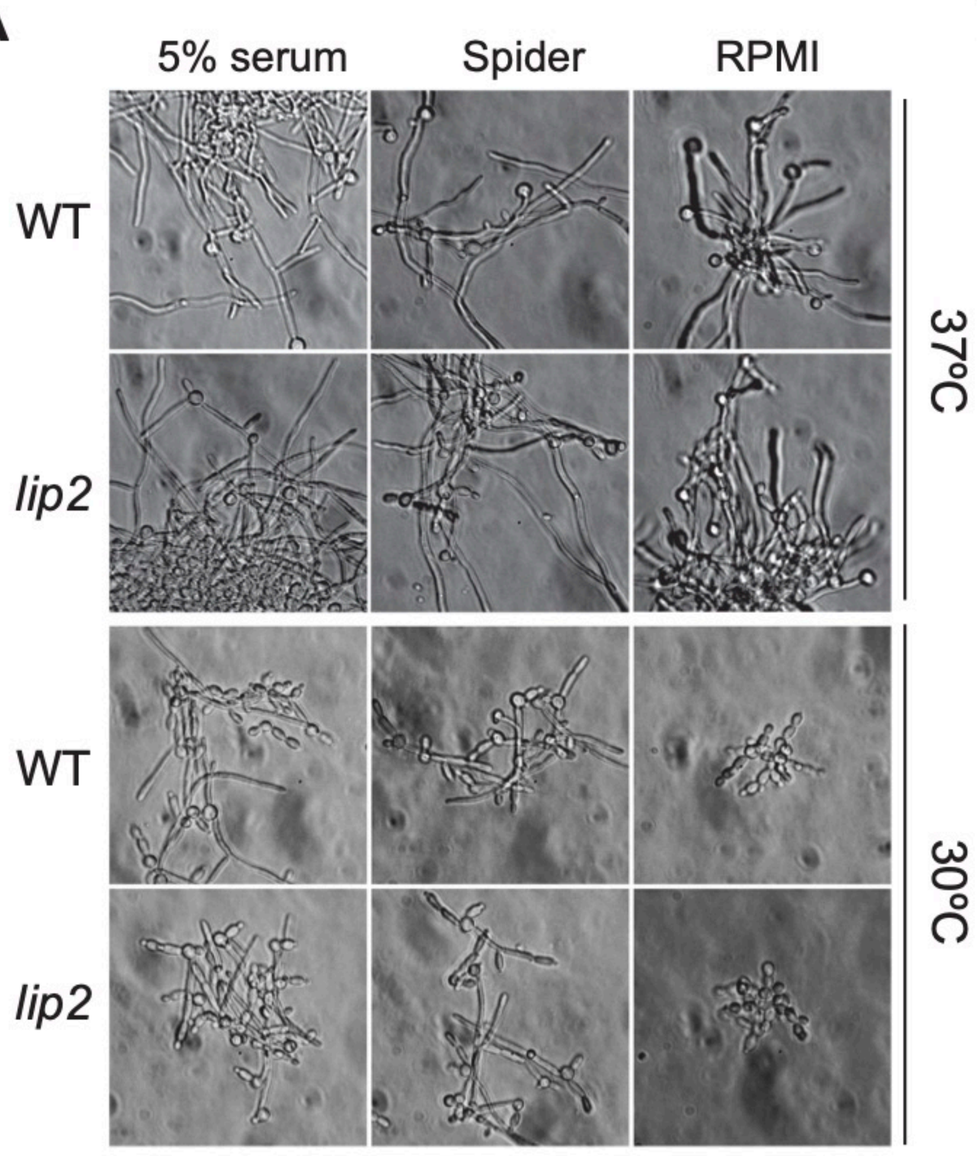
(C) Lip2-catalytic site point mutants phenocopy *lip2* in the coculture assay. BMDCs from C57BL/6J mice were incubated with 0.1 μM chloroform (solvent; dark bars) or 0.1 μM palmitic acid solubilized in chloroform (white bars) and the indicated strains at an MOI of 1 for two hours, followed by measurement of IL-23A in cell supernatants by ELISA. Statistical significance was determined for each mutant between solvent and the PA condition by an unpaired two-tailed t-test; ns: $p > 0.05$; ** $p < 0.001$; **** $p < 0.0001$.

(D) WT *C. albicans* and *lip2* form hyphae in coculture assay, regardless of palmitic acid. BMDCs from C57BL/6J mice were incubated for two hours with solvent alone (dark bars) or 0.1 μM palmitic acid (white bars) in the presence or absence of WT *C. albicans* or *lip2* (MOI of 1). Arrowheads indicate *C. albicans* hyphae

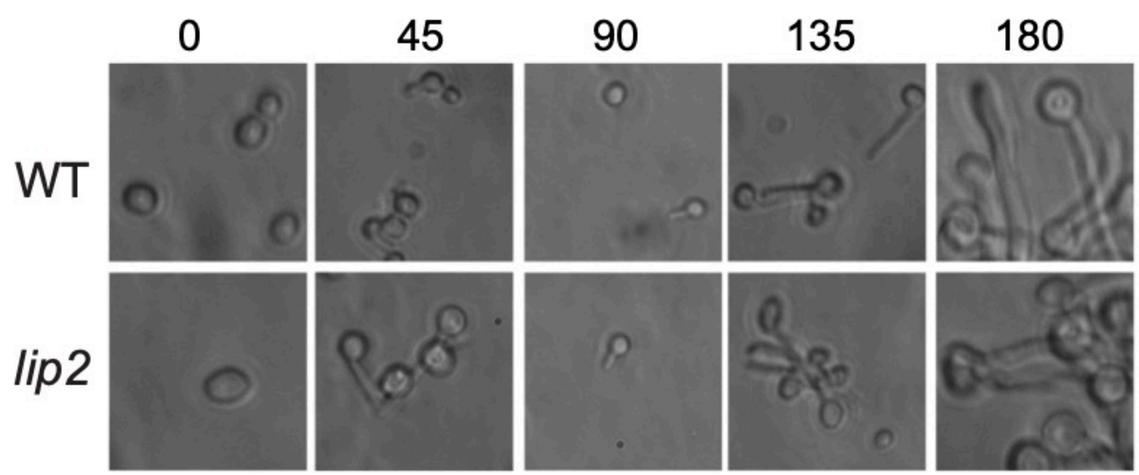
(E) *LIP2* expression is not affected by palmitic acid. WT *C. albicans* was propagated in BMDC culture medium for 2 hours in the presence or absence of palmitic acid or solvent alone, and *LIP2* mRNA was quantified by RT-qPCR (and normalized to *ACT1)*. Statistical significance between different conditions was determined by one-way ANOVA (Tukey's multiple comparisons test); ns: $p > 0.05$.

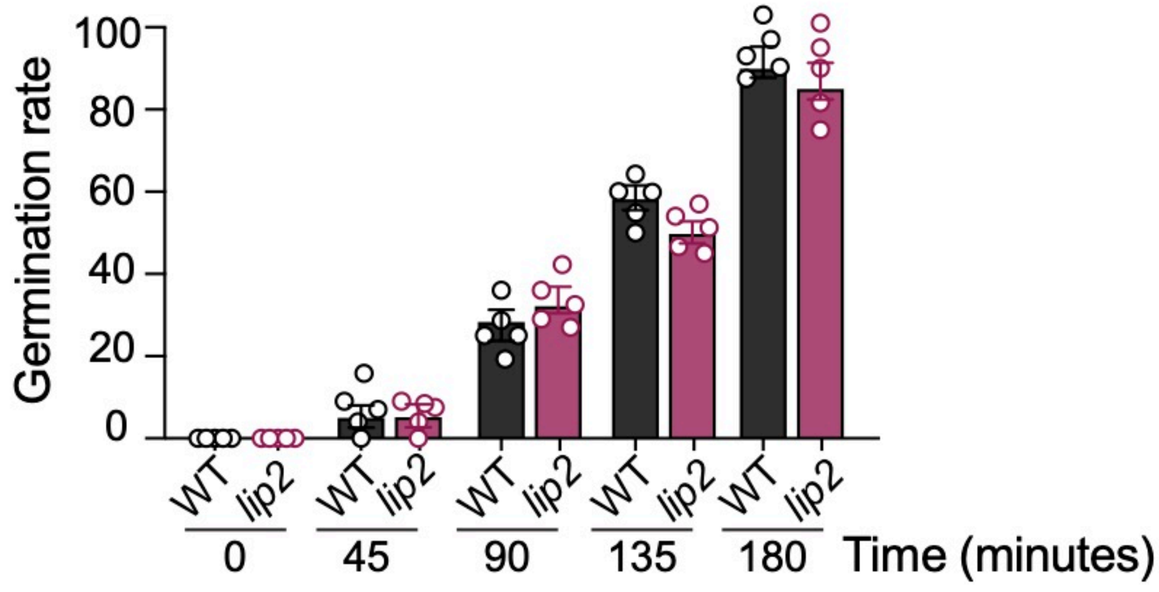
(F) Palmitic acid inhibits BDMC activation by *lip2* but enhances the response to LPS and imiquimod. BMDCs from C57BL/6 mice were incubated for 24 hours with solvent alone (dark bars) or 500 μM palmitic acid (white bars) in the presence or absence of TLR ligands (Pam3CSK4: TLR1/2; Poly(I:C): TLR3; LPS: TLR4; Imiquimod: TLR7/8) or live *C. albicans* (WT or *lip2* at an MOI of 1), followed by measurement of IL-23A in cell supernatants by ELISA. Statistical significance between solvent control and PA condition was determined by an unpaired two-tailed t-test; ns: $p > 0.05$; * $p<0.05$; ** $p<0.001$; *** $p<0.001$.

A

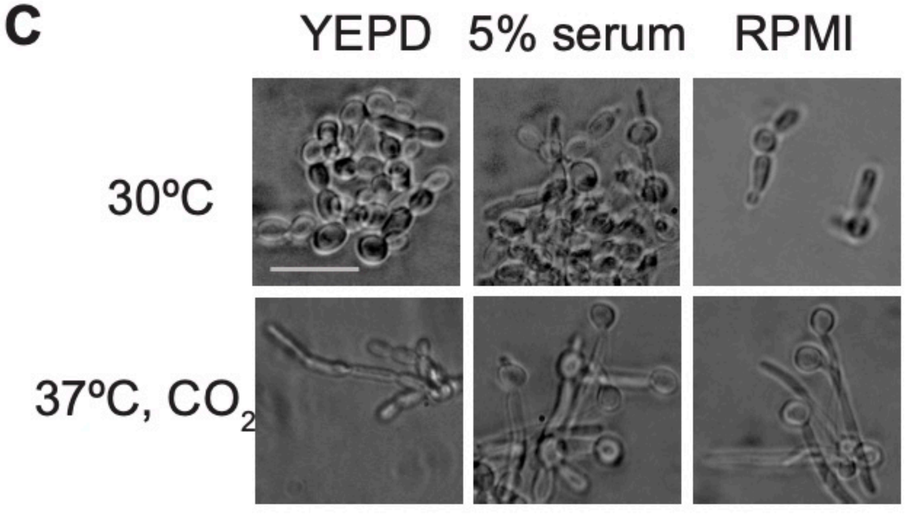

5% serum
Spider
RPMI
WT
lip2
37°C
WT
lip2
30°C

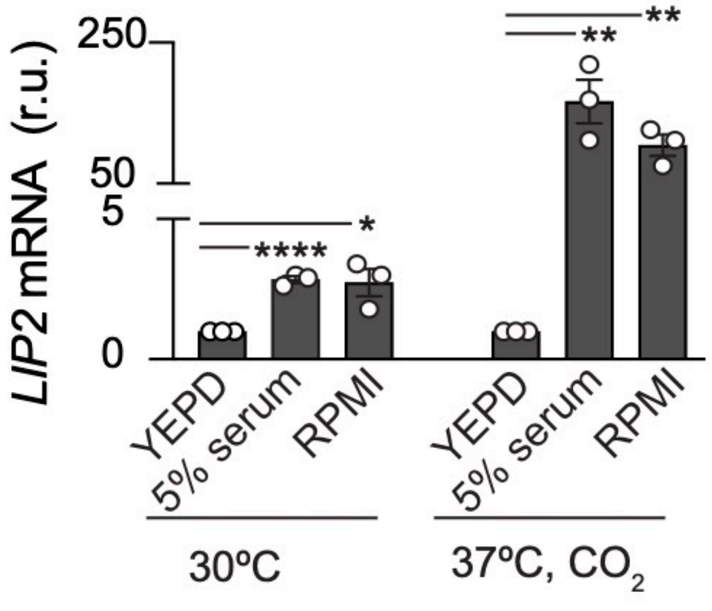
B

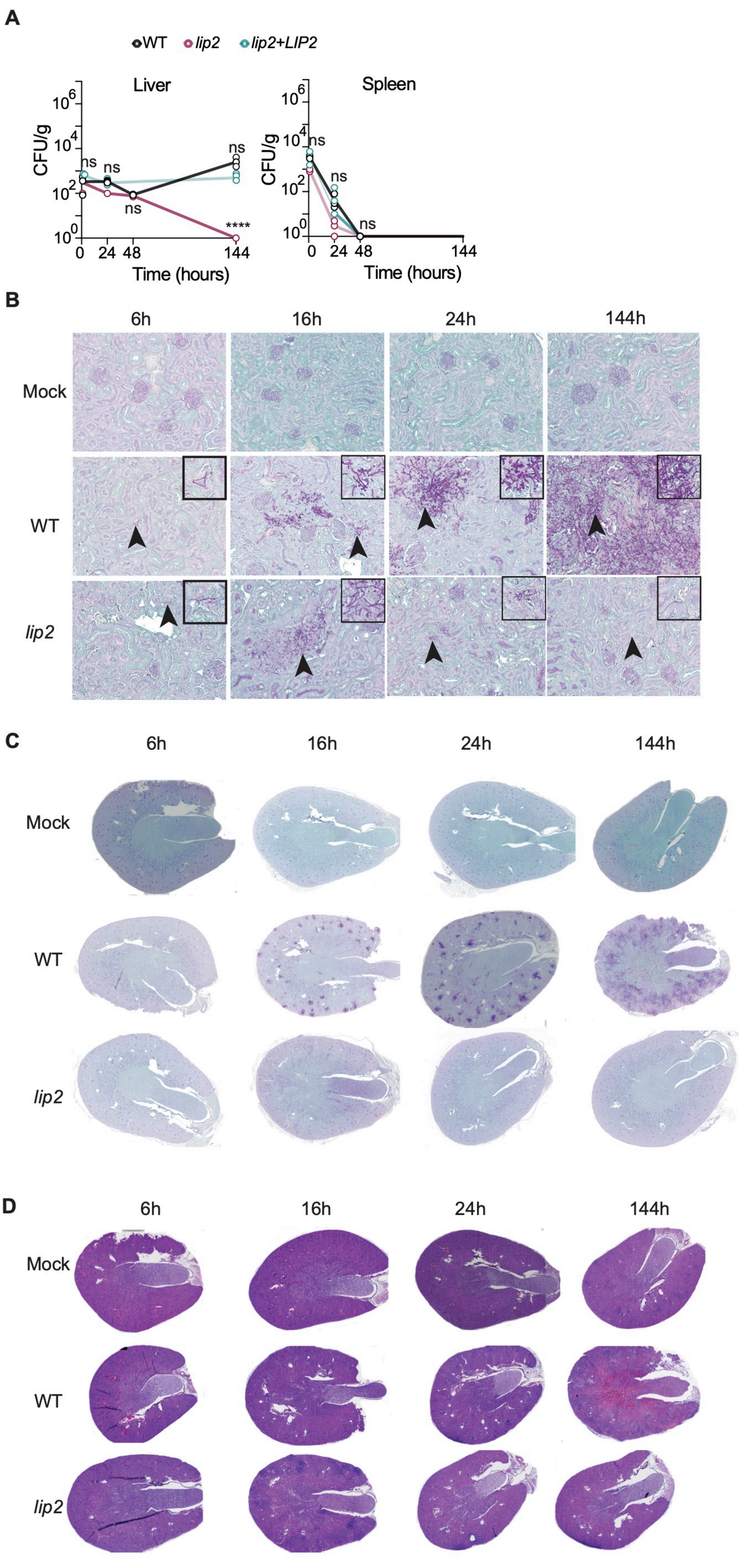

0
45
90
135
180
Minutes
WT
lip2


Germination rate
100
80
60
40
20
0
WT
lip2
0
45
90
135
180
Time (minutes)

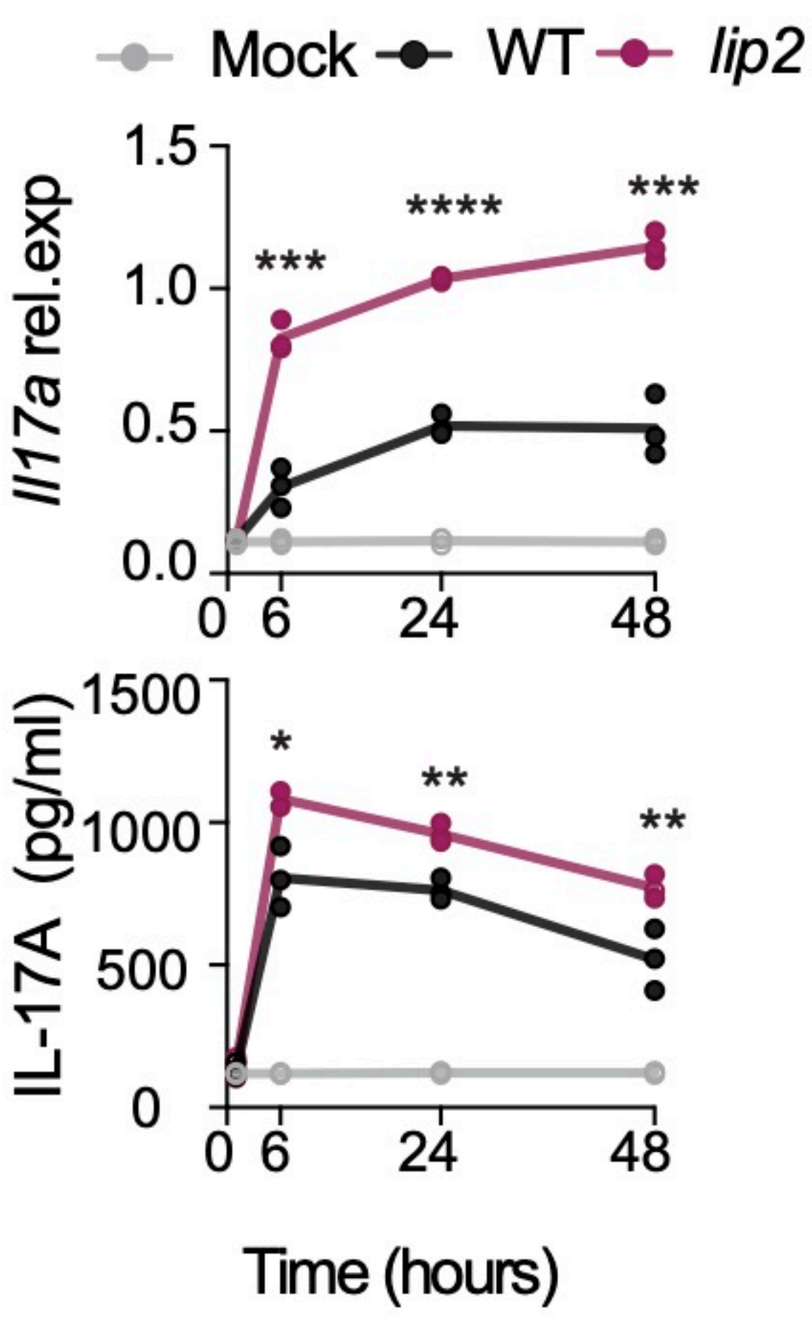
C


YEPD
5% serum
RPMI
30ºC
37ºC, CO2

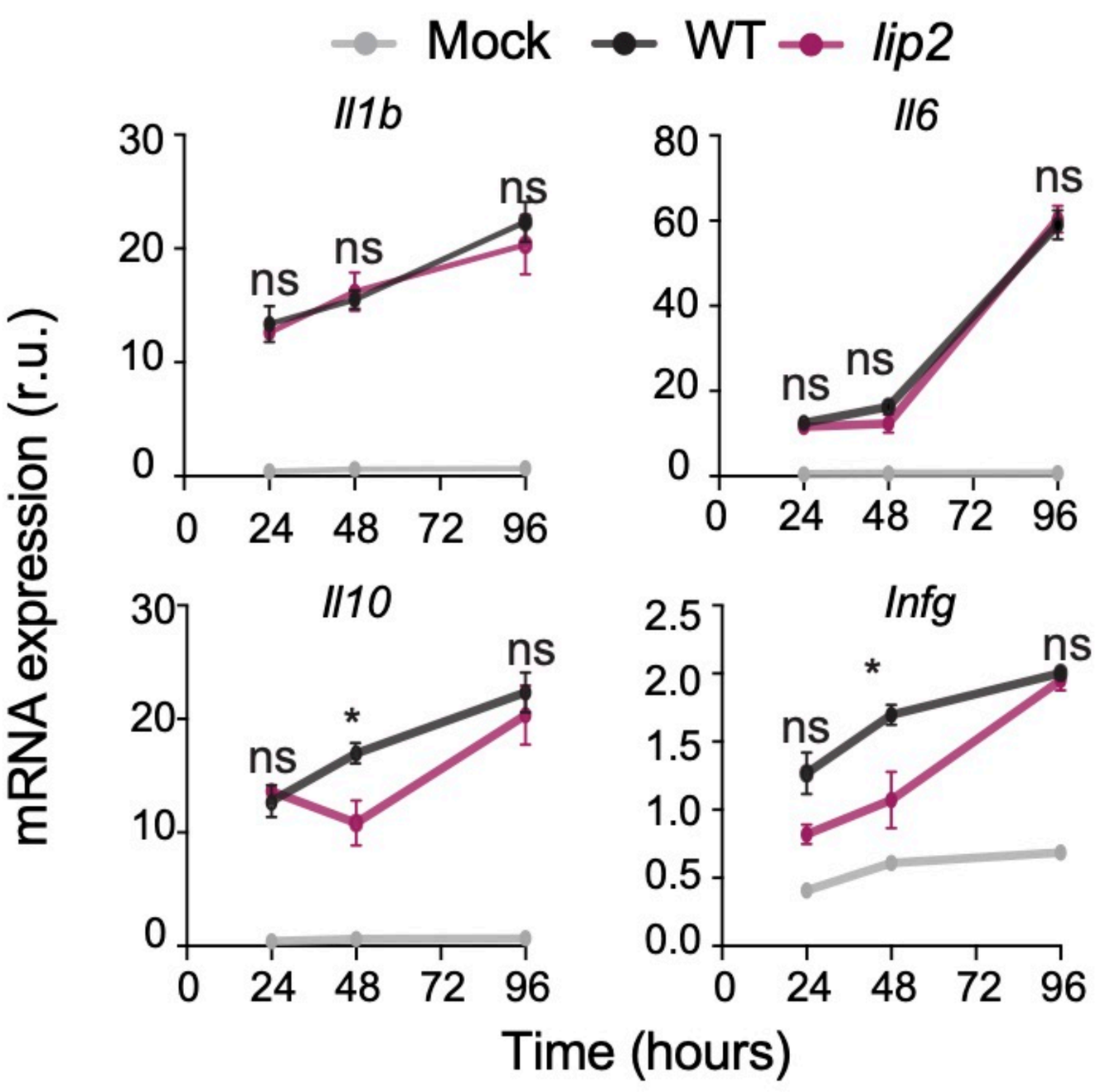

LIP2 mRNA (r.u.)
250
50
5
0
****
*
**
**
YEPD
5% serum
RPMI
30ºC
37ºC, CO2


A
WT
lip2
lip2+LIP2
Liver
Spleen
CFU/g
Time (hours)
ns
****
B
6h
16h
24h
144h
Mock
WT
lip2
C
D

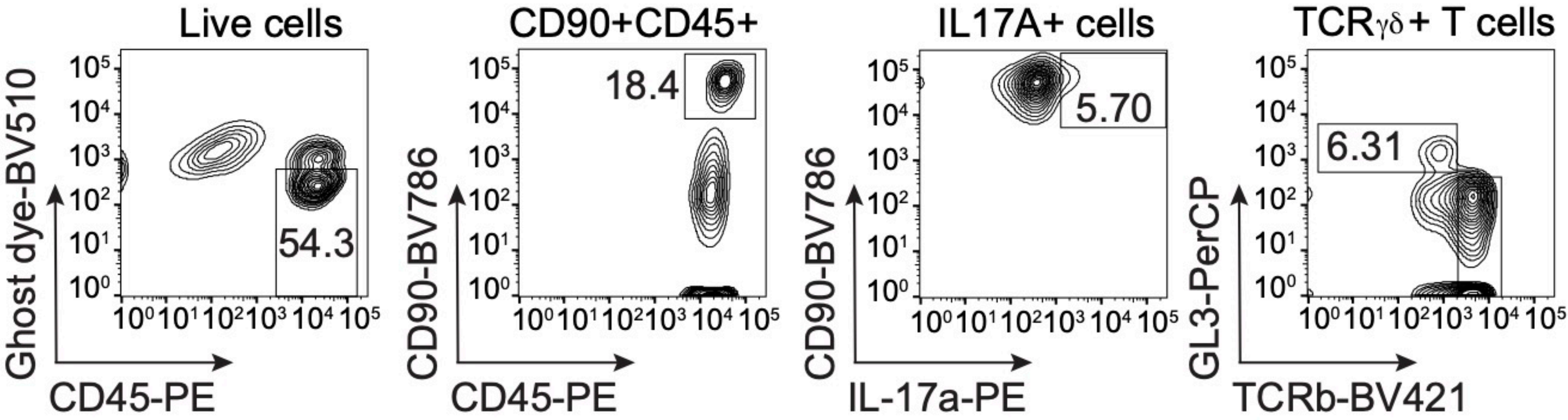
A


Mock
WT
lip2
Il17a rel.exp
1.5
1.0
0.5
0.0
0
6
24
48
***
****
***
IL-17A (pg/ml)
1500
1000
500
0
*
**
**
Time (hours)

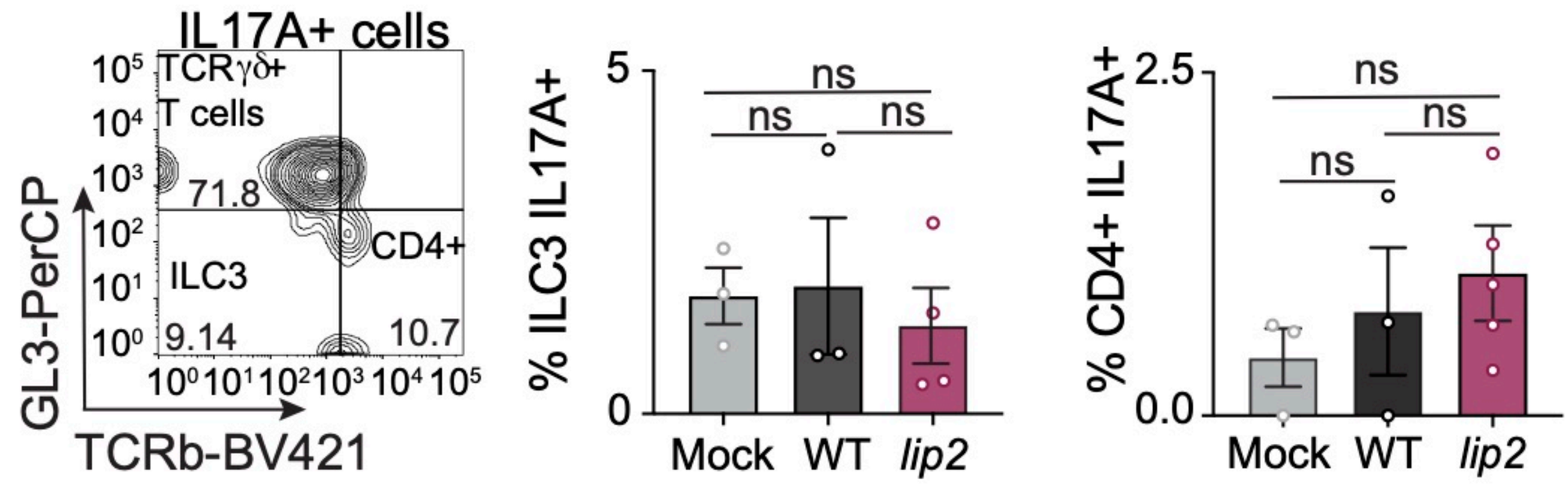
B


Mock
WT
lip2
Il1b
Il6
Il10
Infg
mRNA expression (r.u.)
30
20
10
0
80
60
40
20
2.5
2.0
1.5
1.0
0.5
0.0
0
24
48
72
96
ns
*
Time (hours)

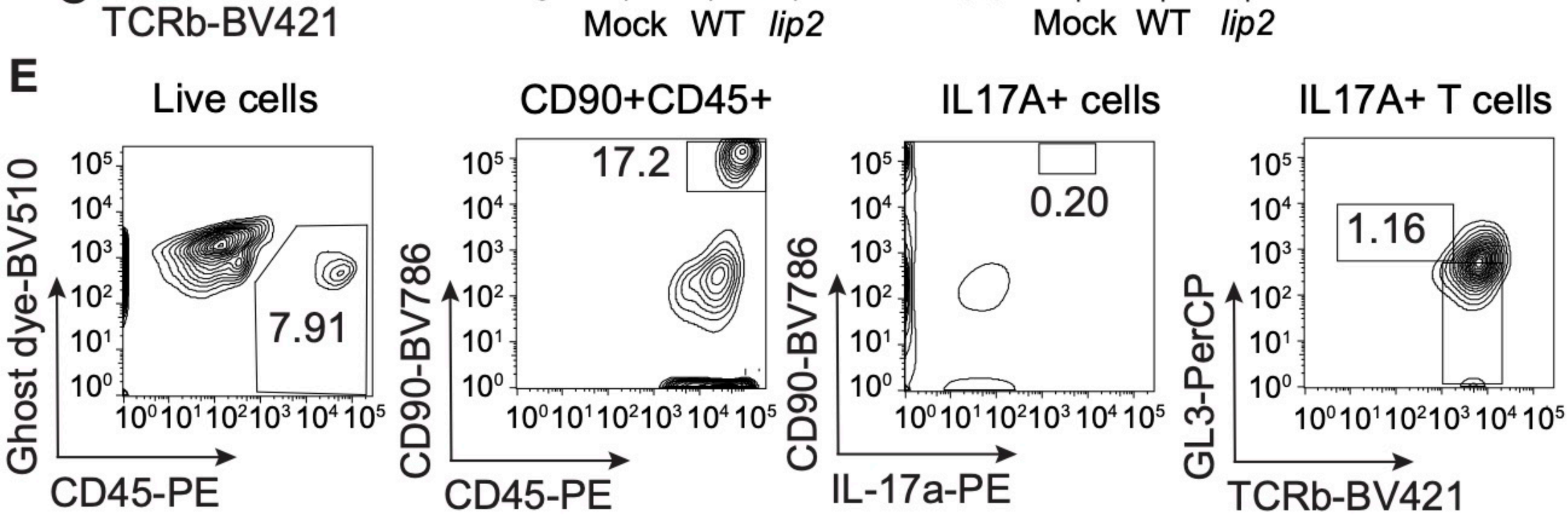
C


Live cells
CD90+CD45+
IL17A+ cells
IL17A+ TCRγδ+ T cells
Ghost dye-BV510
CD45-PE
54.3
CD90-BV786
18.4
IL-17a-PE
5.70
GL3-PerCP
TCRb-BV421
6.31

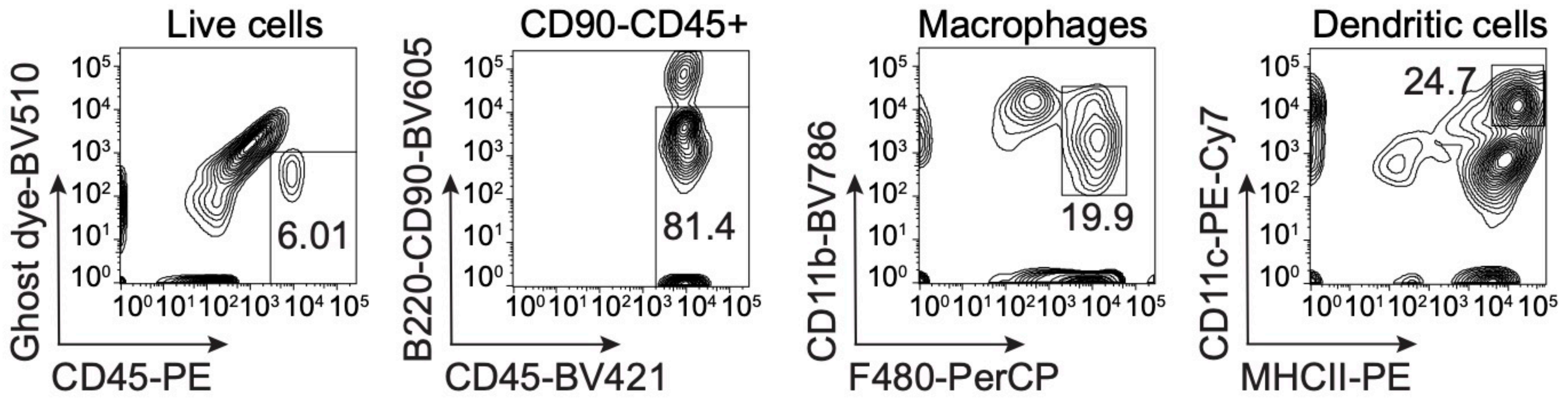
D


IL17A+ cells
TCRγδ+ T cells
ILC3
CD4+
71.8
9.14
10.7
GL3-PerCP
TCRb-BV421
% ILC3 IL17A+
% CD4+ IL17A+
ns
Mock
WT
lip2

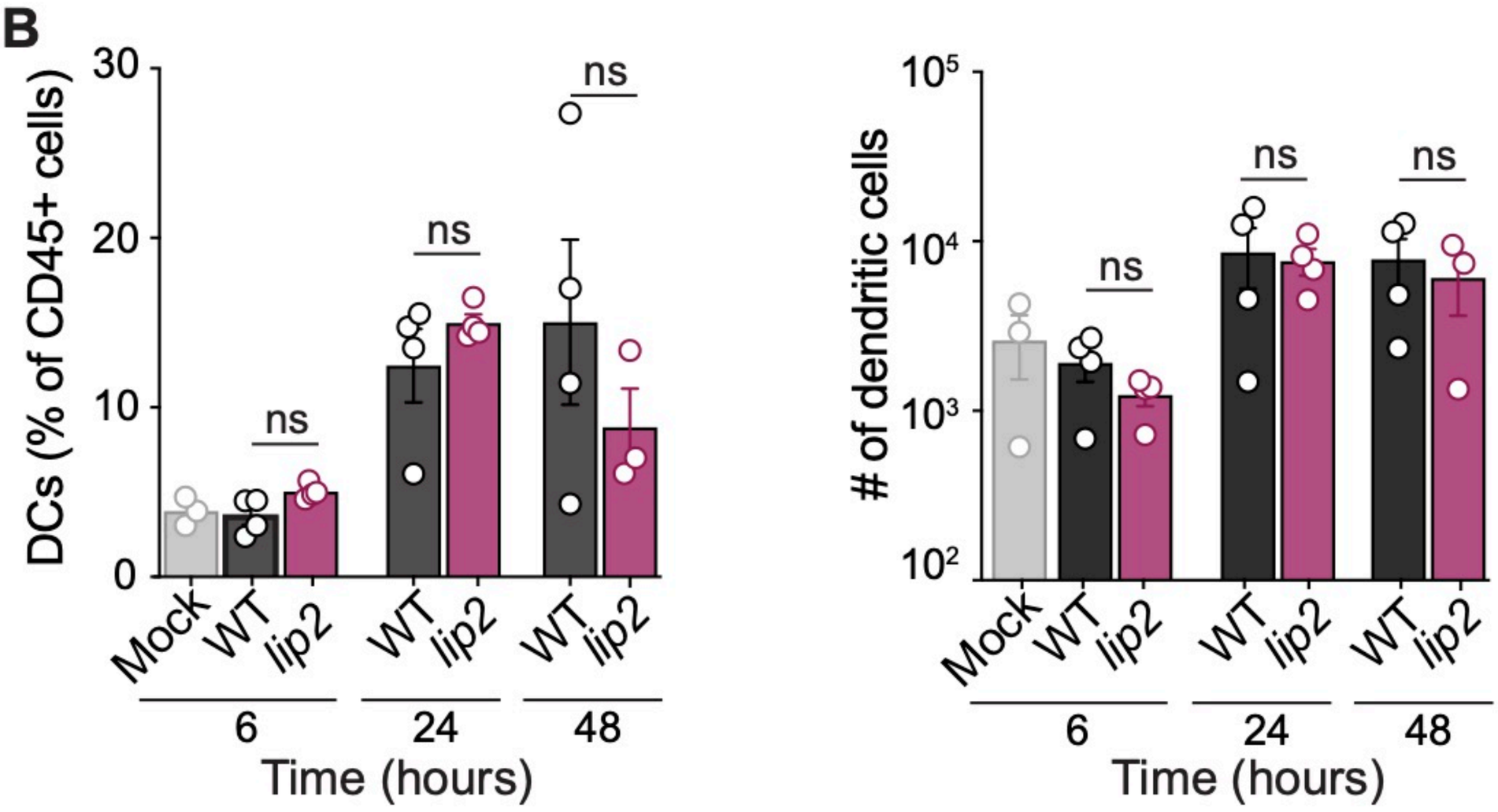
E


Live cells
CD90+CD45+
IL17A+ cells
IL17A+ T cells
Ghost dye-BV510
CD45-PE
7.91
CD90-BV786
17.2
IL-17a-PE
0.20
GL3-PerCP
TCRb-BV421
1.16
TCRγδ+ T cells
IL17A+
GL3-PerCP
IL17A-PE
3.68
% γδ T cells IL17A+
*
ns
Mock
WT
lip2

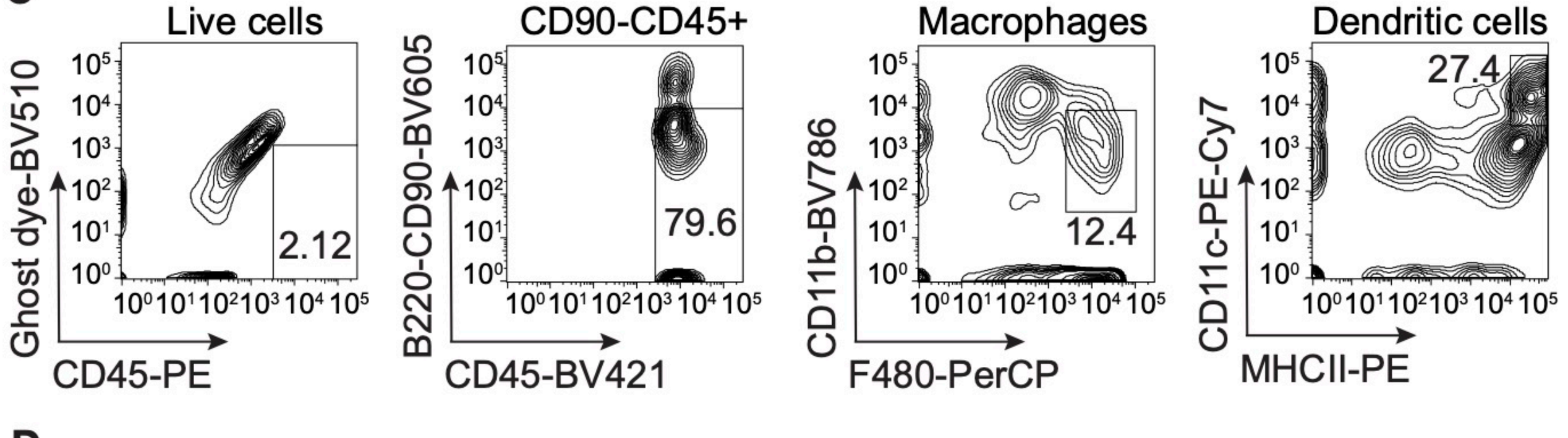
A


Live cells
Ghost dye-BV510
CD45-PE
6.01
CD90-CD45+
B220-CD90-BV605
CD45-BV421
81.4
Macrophages
CD11b-BV786
F480-PerCP
19.9
Dendritic cells
CD11c-PE-Cy7
MHCII-PE
24.7

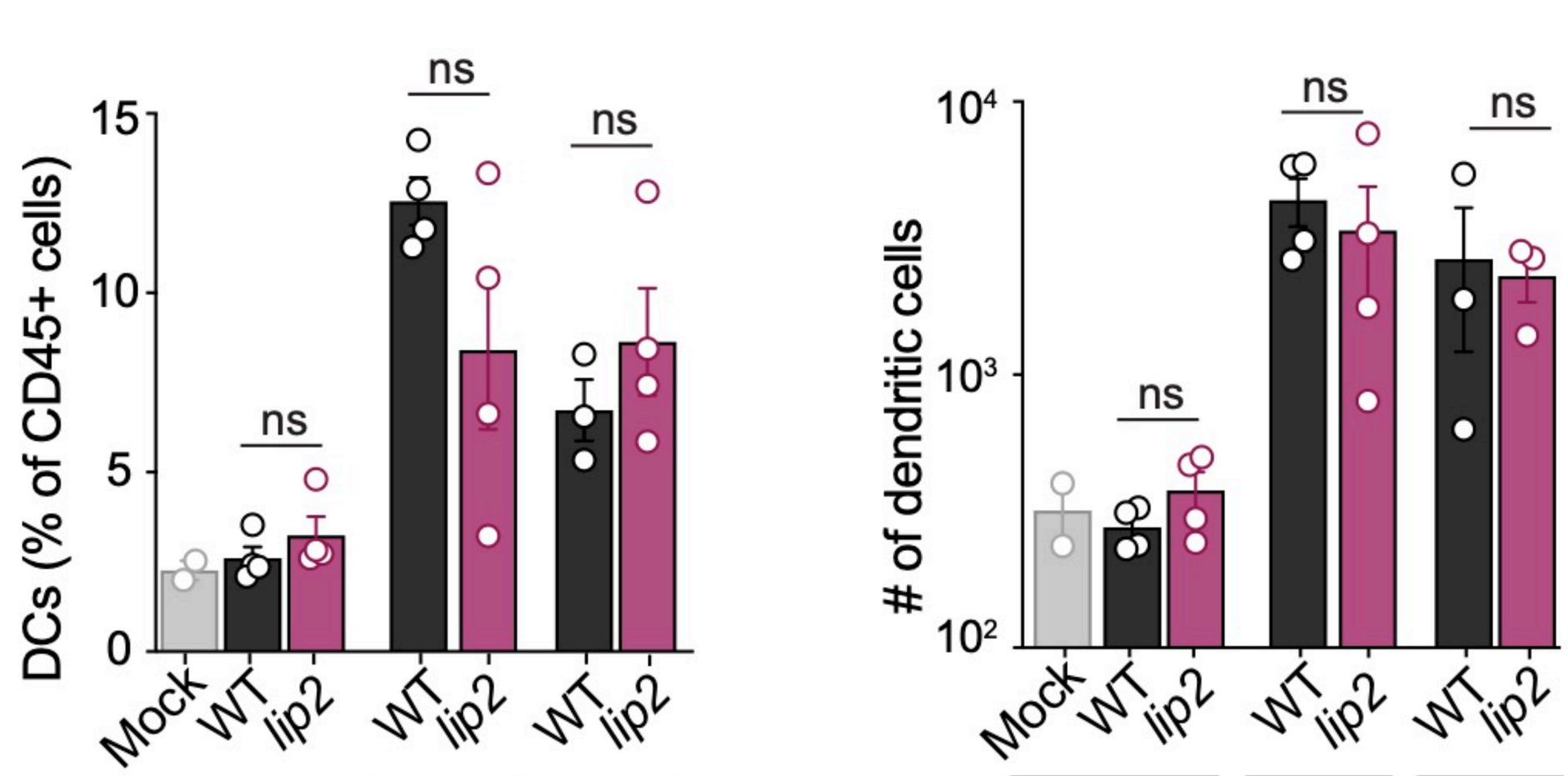
B


DCs (% of CD45+ cells)
ns
Mock
WT
lip2
6
24
48
Time (hours)
# of dendritic cells
Time (hours)

C

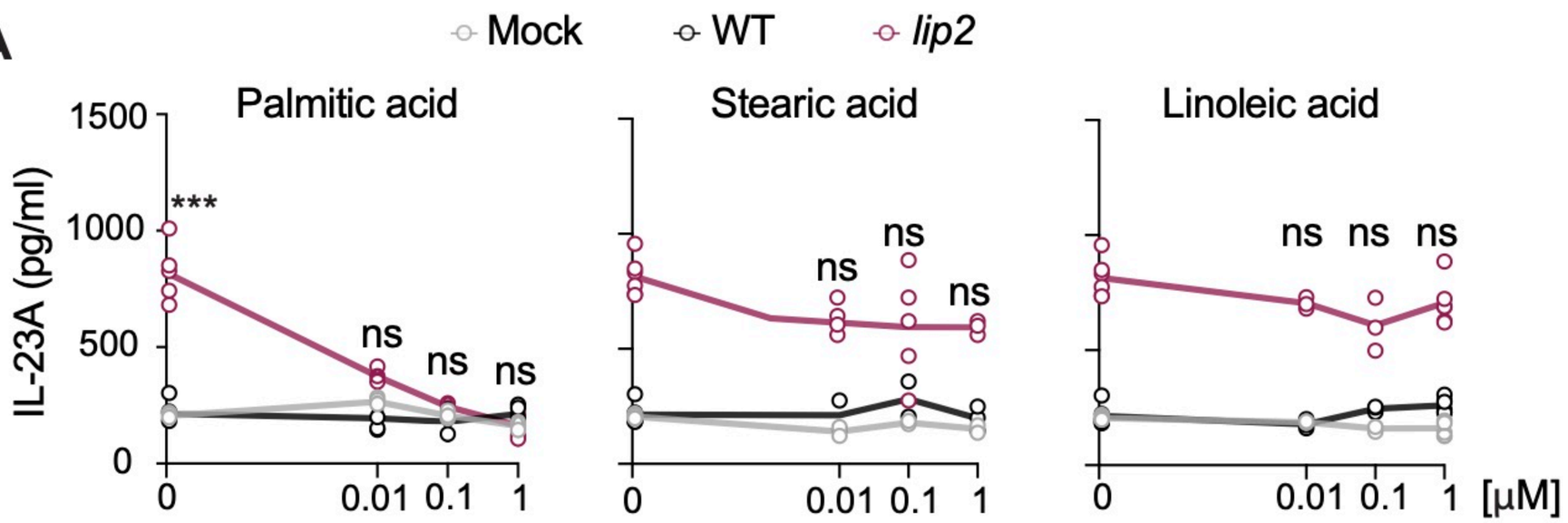

Live cells
Ghost dye-BV510
CD45-PE
2.12
CD90-CD45+
B220-CD90-BV605
CD45-BV421
79.6
Macrophages
CD11b-BV786
F480-PerCP
12.4
Dendritic cells
CD11c-PE-Cy7
MHCII-PE
27.4

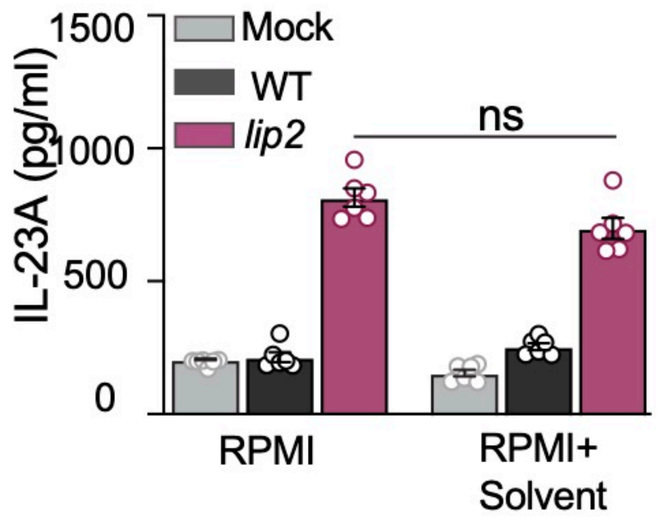
D

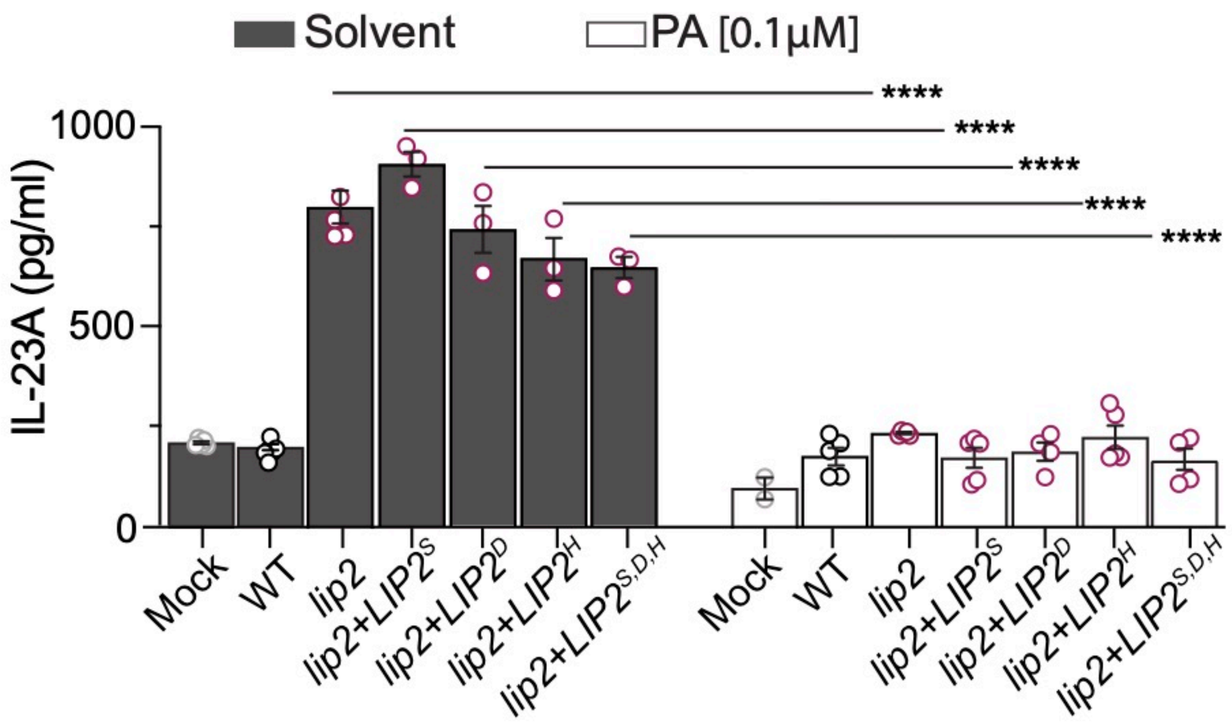

DCs (% of CD45+ cells)
ns
Mock
WT
lip2
6
24
48
Time (hours)
# of dendritic cells
Time (hours)

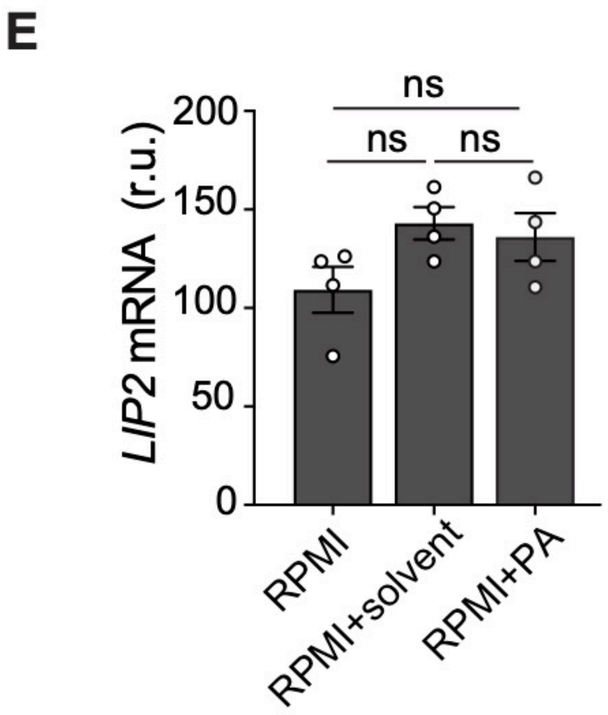
E

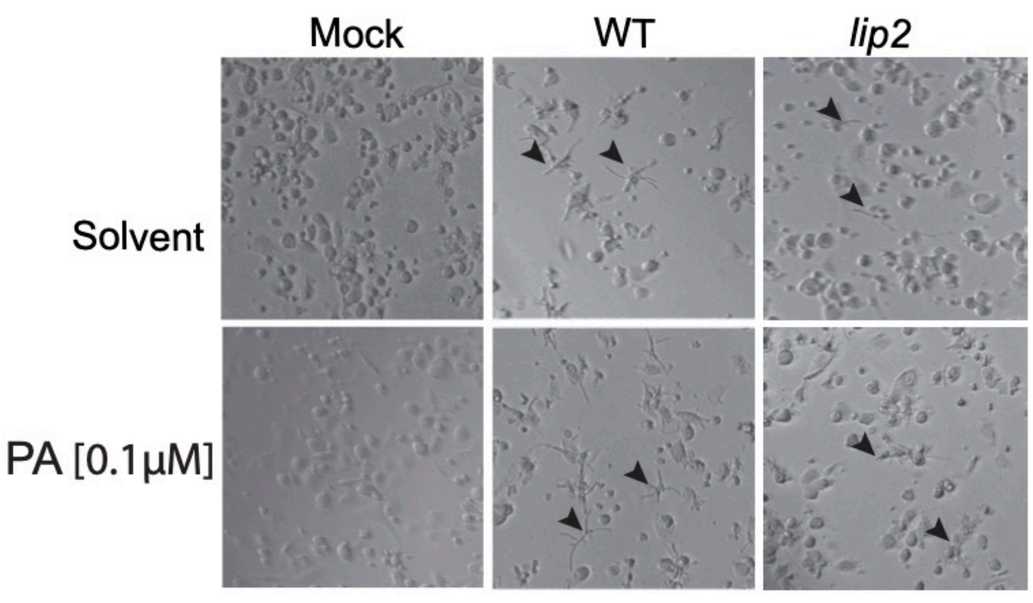
Relative expression / ACT1
LIP2
ECE1
EFG1
SAP6
LIP10

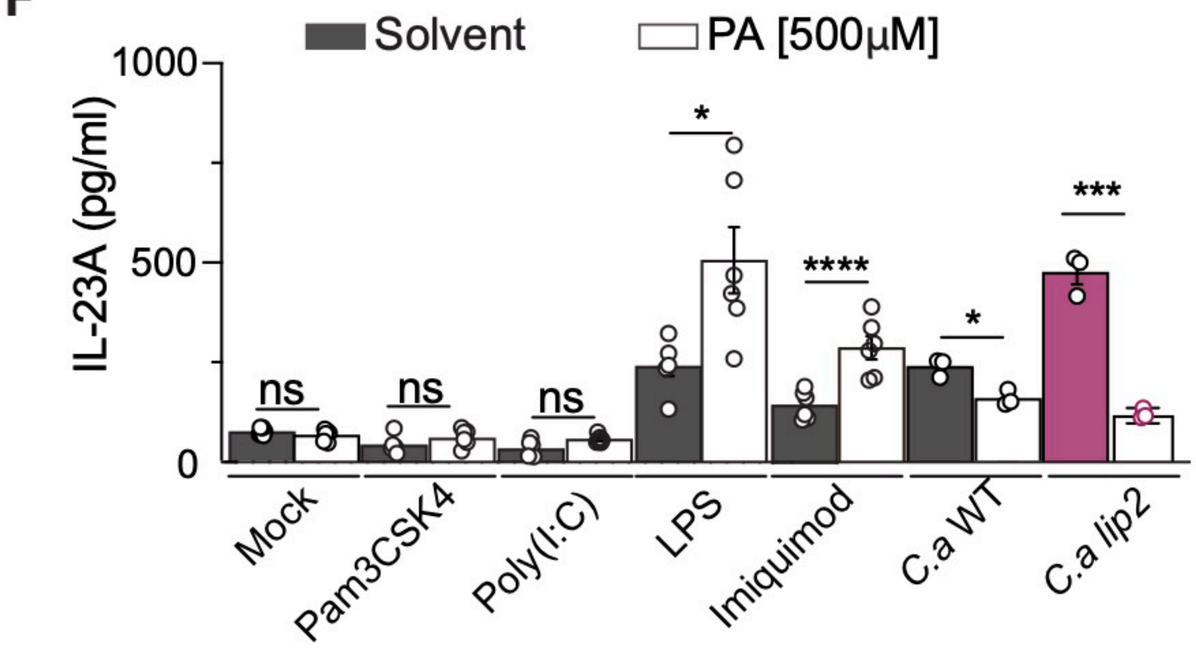

A
Mock
WT
lip2
Palmitic acid
Stearic acid
Linoleic acid
IL-23A (pg/ml)
1500
1000
500
0
***
ns
ns
ns
ns
ns
ns
ns
ns
ns
0
0.01
0.1
1
[µM]


B
Mock
WT
lip2
IL-23A (pg/ml)
1500
1000
500
0
ns
RPMI
RPMI+
Solvent


C
Solvent
PA [0.1µM]
IL-23A (pg/ml)
1000
500
0
****
****
****
****
****
Mock
WT
lip2
lip2+LIP2S
lip2+LIP2D
lip2+LIP2H
lip2+LIP2S,D,H


E
LIP2 mRNA (r.u.)
200
150
100
50
0
ns
ns
ns
RPMI
RPMI+solvent
RPMI+PA


D
Mock
WT
lip2
Solvent
PA [0.1µM]


F
Solvent
PA [500µM]
IL-23A (pg/ml)
1000
500
0
ns
ns
ns
*
****
*
***
Mock
Pam3CSK4
Poly(I:C)
LPS
Imiquimod
C.a WT
C.a lip2