Figure 1

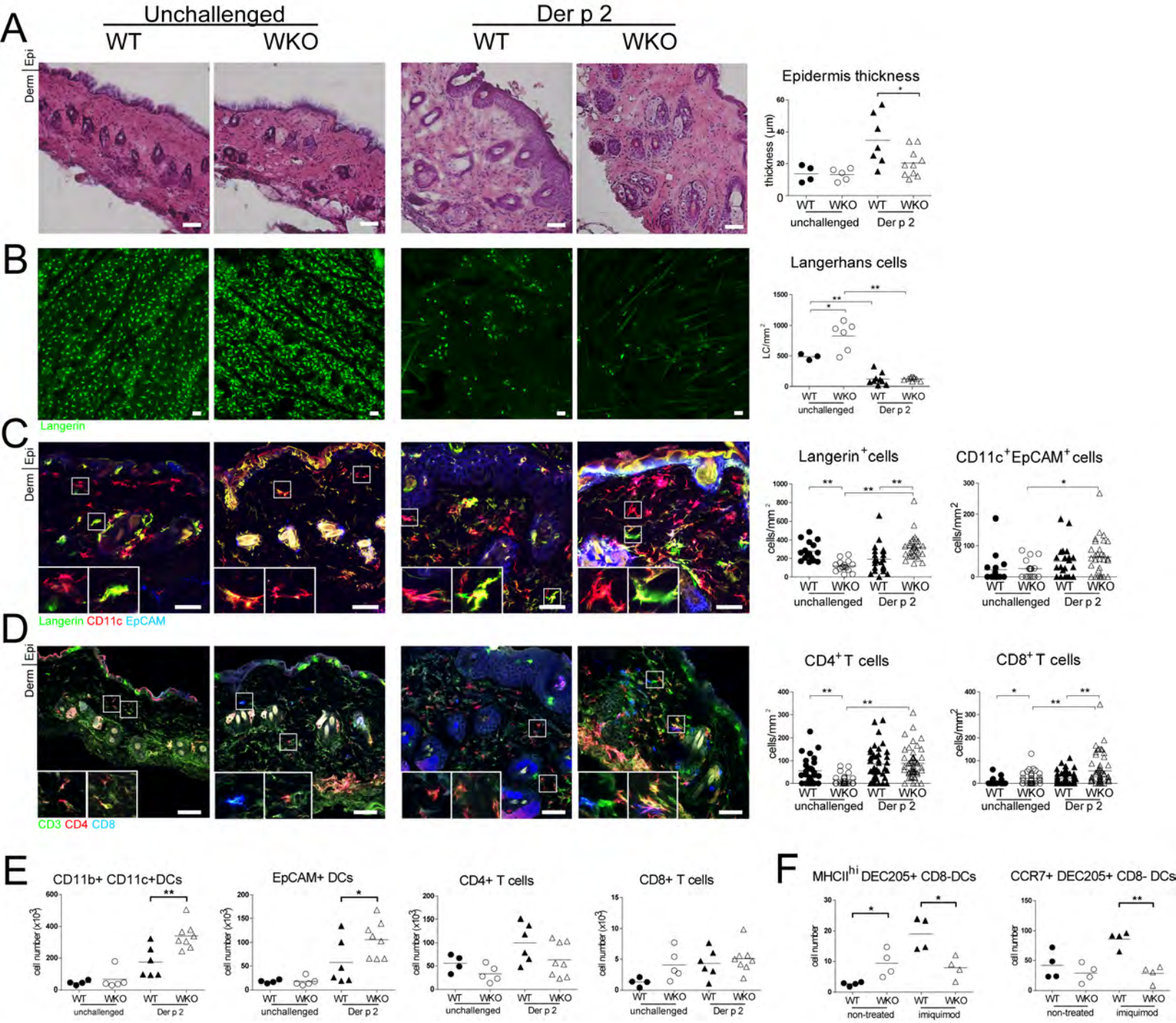

A
Unchallenged
Der p 2
WT
WKO
Epi
Derm
Epidermis thickness
thickness (µm)
unchallenged
Der p 2
B
Langerin
Langerhans cells
LC/mm²
C
Langerin
CD11c
EpCAM
Langerin⁺cells
CD11c⁺EpCAM⁺ cells
cells/mm²
D
CD3
CD4
CD8
CD4⁺ T cells
CD8⁺ T cells
E
CD11b+ CD11c+DCs
EpCAM+ DCs
CD4+ T cells
CD8+ T cells
cell number (x10³)
F
MHCII^hi DEC205+ CD8-DCs
CCR7+ DEC205+ CD8- DCs
cell number
non-treated
imiquimod

Figure 2

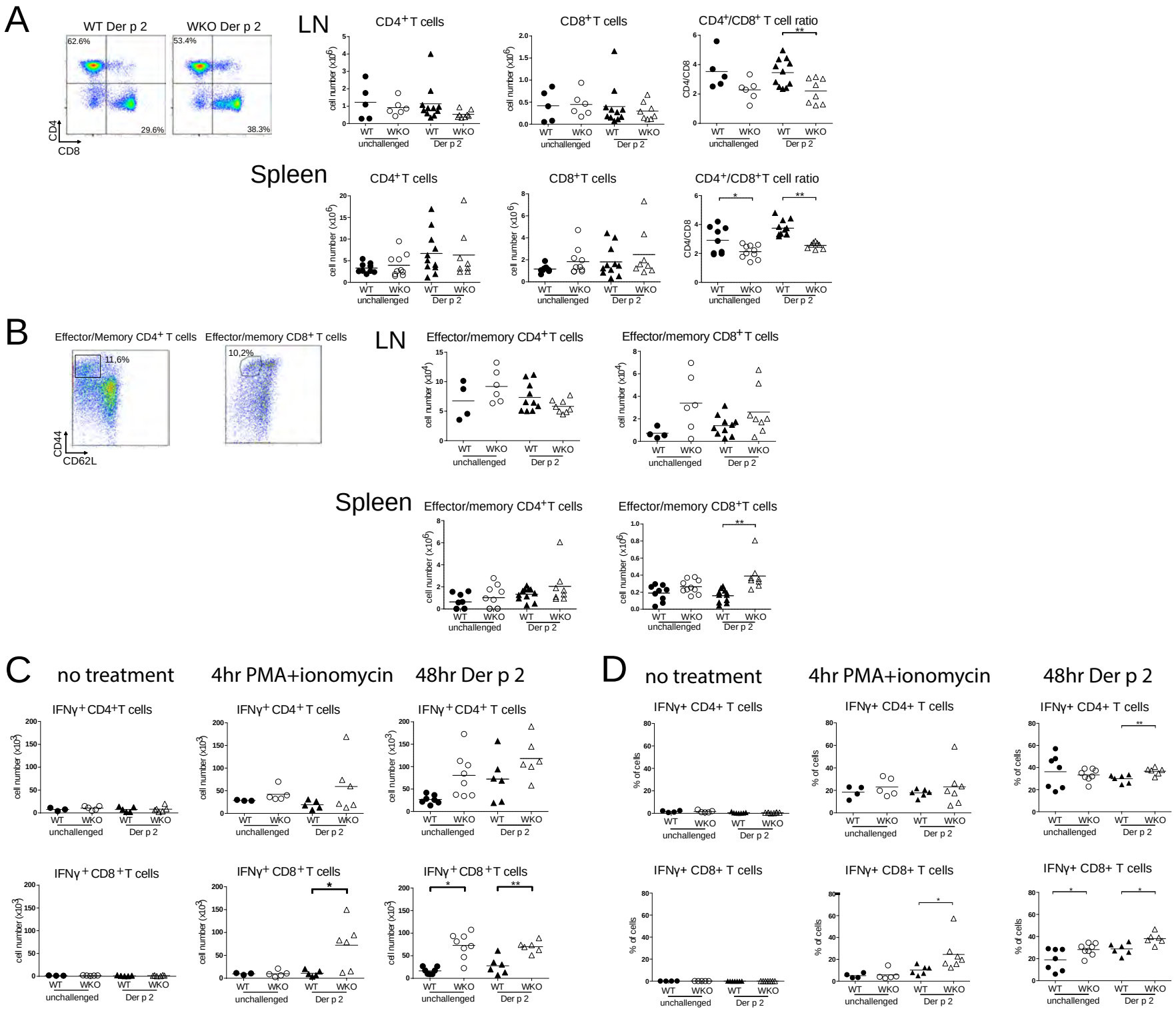

A
WT Der p 2
WKO Der p 2
62.6%
29.6%
53.4%
38.3%
CD4
CD8
LN
CD4+ T cells
CD8+ T cells
CD4+/CD8+ T cell ratio
cell number (x10^6)
CD4/CD8
WT
WKO
unchallenged
Der p 2
Spleen
B
Effector/Memory CD4+ T cells
Effector/memory CD8+ T cells
11,6%
10,2%
CD44
CD62L
Effector/memory CD4+T cells
Effector/memory CD8+T cells
cell number (x10^4)
C
no treatment
4hr PMA+ionomycin
48hr Der p 2
IFNγ+CD4+T cells
IFNγ+CD8+T cells
cell number (x10^3)
D
IFNγ+ CD4+ T cells
IFNγ+ CD8+ T cells
% of cells

Figure 3

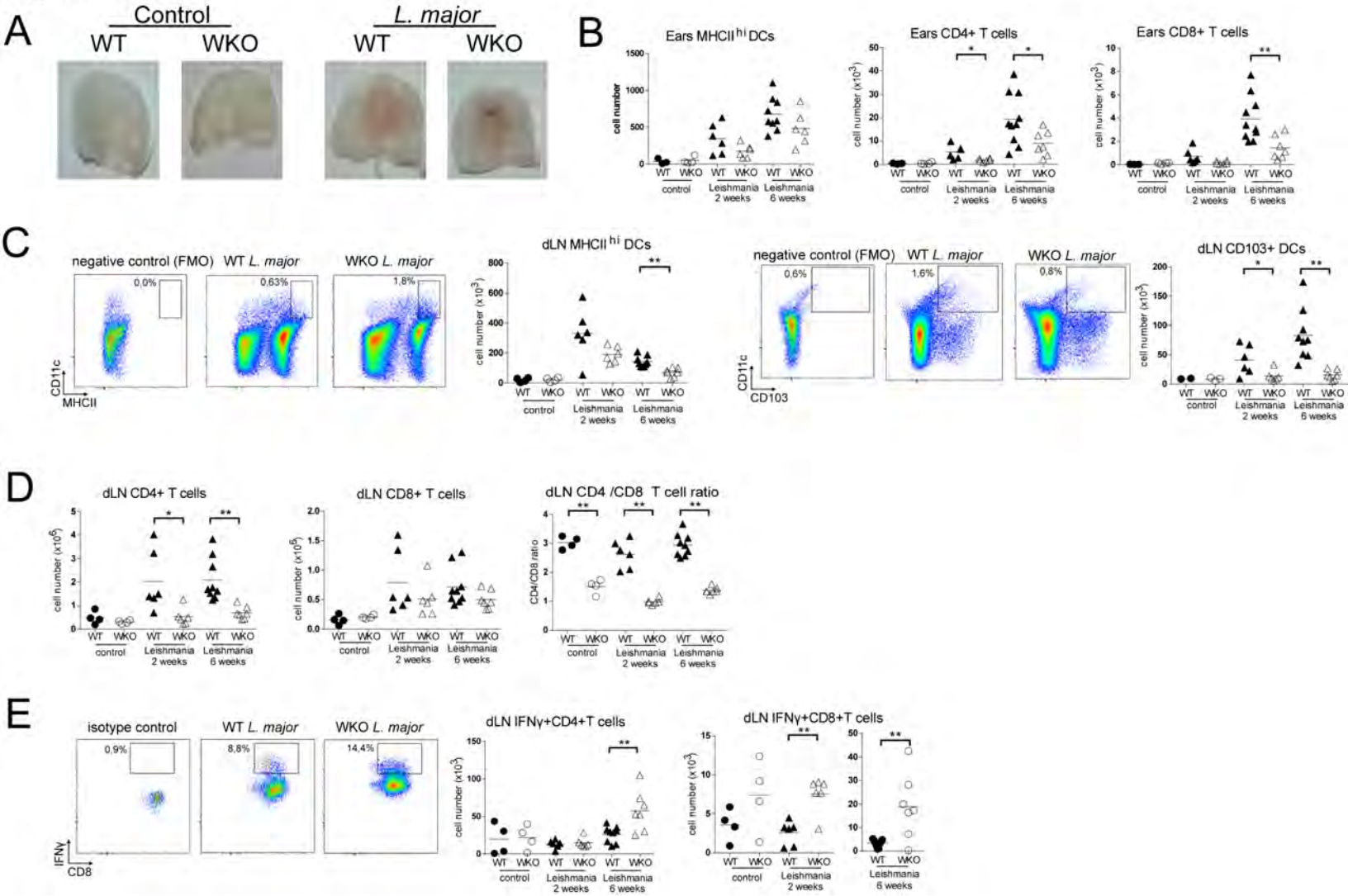

A
Control
WT
WKO
L. major
WT
WKO
B
Ears MHCII hi DCs
cell number
Ears CD4+ T cells
cell number (x10³)
Ears CD8+ T cells
cell number (x10³)
WT WKO WT WKO WT WKO
control
Leishmania 2 weeks
Leishmania 6 weeks
C
negative control (FMO)
WT L. major
WKO L. major
0,0%
0,63%
1,8%
CD11c
MHCII
dLN MHCII hi DCs
cell number (x10³)
negative control (FMO)
WT L. major
WKO L. major
0,6%
1,6%
0,8%
CD11c
CD103
dLN CD103+ DCs
cell number (x10³)
D
dLN CD4+ T cells
cell number (x10⁶)
dLN CD8+ T cells
cell number (x10⁶)
dLN CD4 /CD8 T cell ratio
CD4/CD8 ratio
E
isotype control
WT L. major
WKO L. major
0,9%
8,8%
14,4%
IFNγ
CD8
dLN IFNγ+CD4+T cells
cell number (x10³)
dLN IFNγ+CD8+T cells
cell number (x10³)

Figure 4

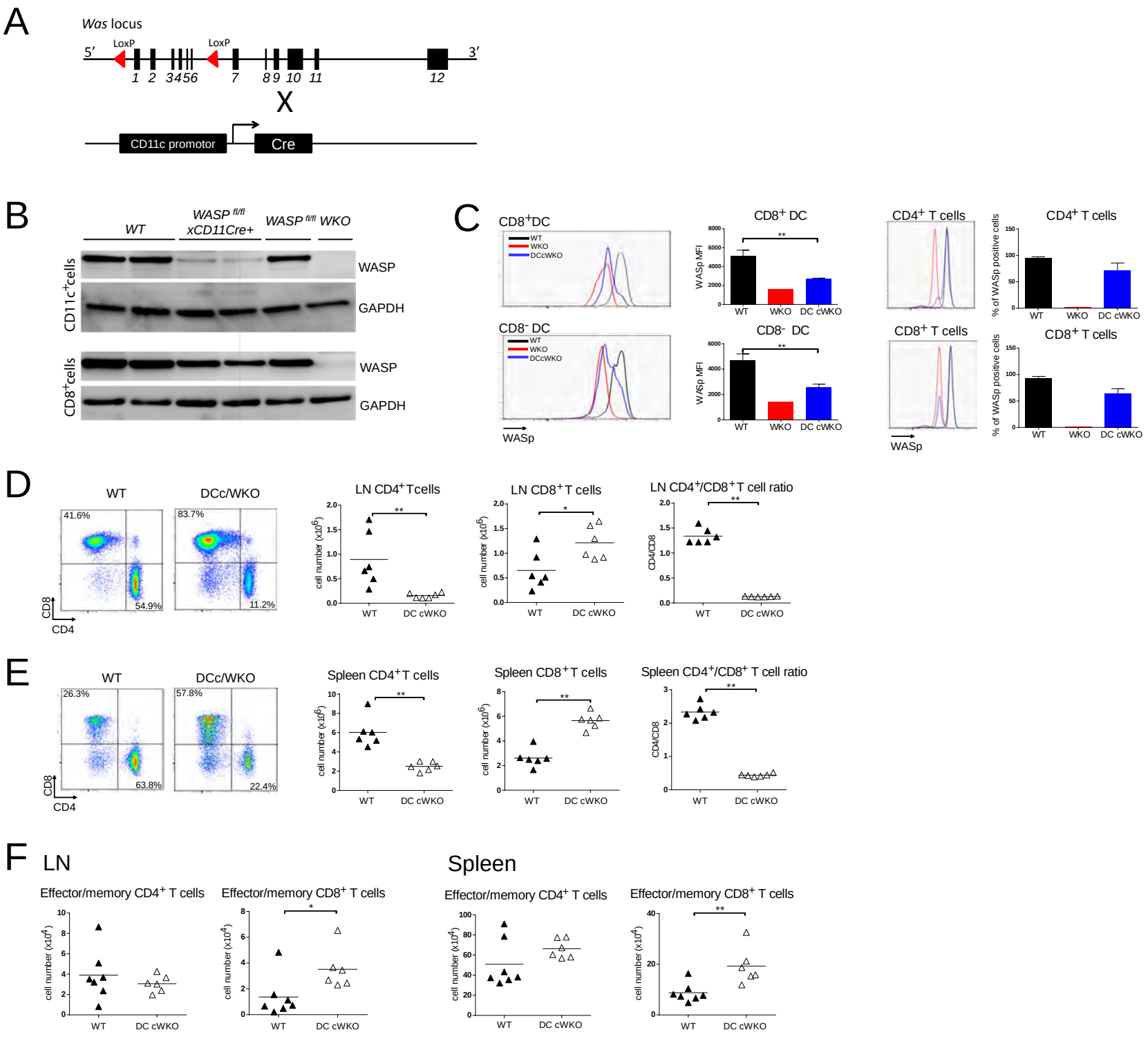

A
Was locus
5'
LoxP
LoxP
3'
1 2 3456 7 89 10 11 12
X
CD11c promotor
Cre
B
WT
WASP fl/fl xCD11Cre+
WASP fl/fl
WKO
CD11c+cells
WASP
GAPDH
CD8+cells
WASP
GAPDH
C
CD8+DC
WT
WKO
DCcWKO
WASp
CD8+ DC
WASp MFI
WT
WKO
DC cWKO
CD4+ T cells
% of WASp positive cells
CD8- DC
CD8+ T cells
D
WT
DCc/WKO
41.6%
54.9%
83.7%
11.2%
CD8
CD4
LN CD4+Tcells
LN CD8+T cells
LN CD4+/CD8+T cell ratio
cell number (x10^6)
CD4/CD8
E
WT
DCc/WKO
26.3%
63.8%
57.8%
22.4%
Spleen CD4+T cells
Spleen CD8+T cells
Spleen CD4+/CD8+ T cell ratio
F
LN
Effector/memory CD4+ T cells
Effector/memory CD8+ T cells
cell number (x10^4)
Spleen
Effector/memory CD4+ T cells
Effector/memory CD8+ T cells

Figure 5

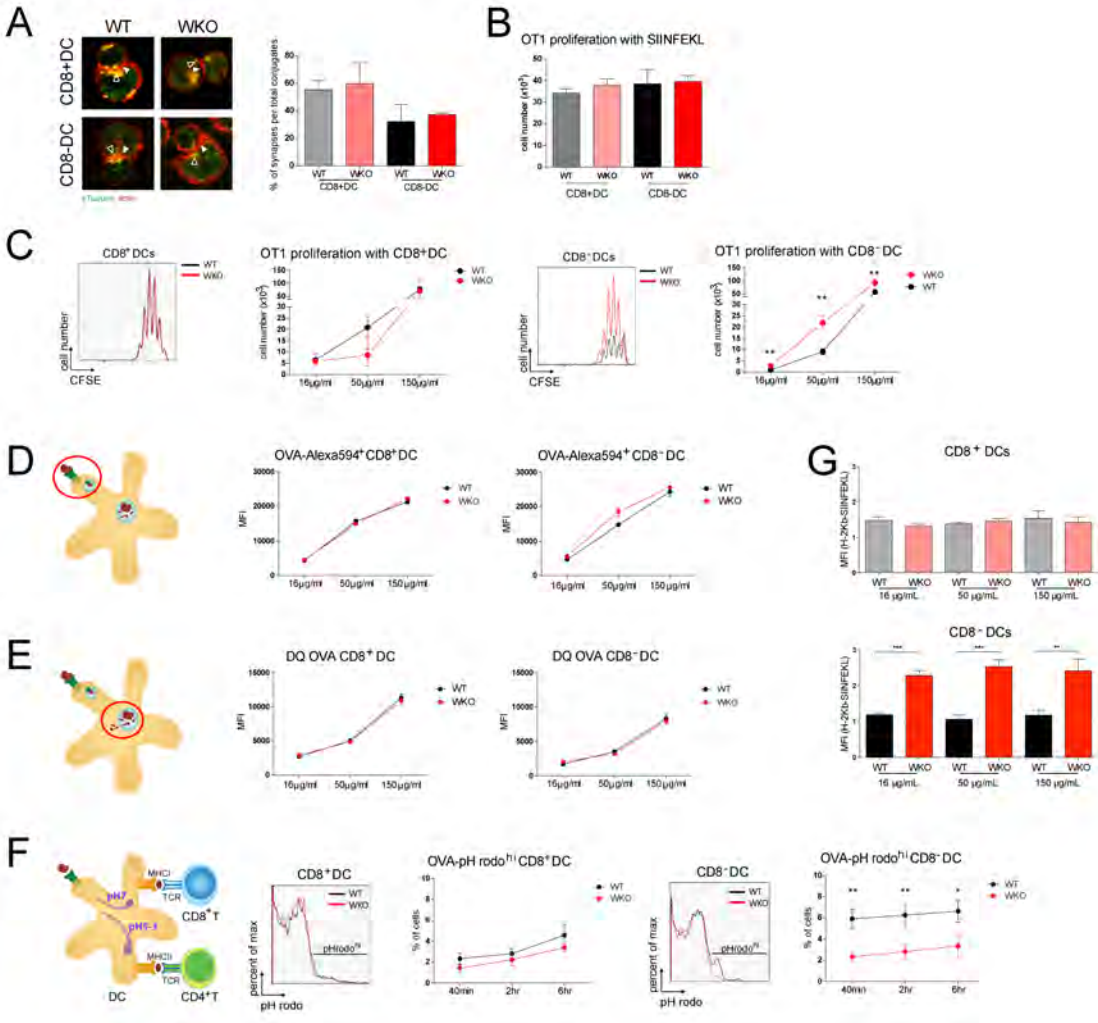

A
WT
WKO
CD8+DC
CD8-DC
% of synapses per total conjugates
CD8+DC
CD8-DC
B
OT1 proliferation with SIINFEKL
cell number (x10³)
C
CD8⁺DCs
cell number
CFSE
OT1 proliferation with CD8⁺DC
CD8⁻DCs
OT1 proliferation with CD8⁻DC
16μg/ml
50μg/ml
150μg/ml
D
OVA-Alexa594⁺CD8⁺DC
OVA-Alexa594⁺CD8⁻DC
MFI
E
DQ OVA CD8⁺DC
DQ OVA CD8⁻DC
F
MHCI
TCR
CD8⁺T
pH7
pH5.5
MHCII
DC
CD4⁺T
CD8⁺DC
percent of max
pH rodo
OVA-pH rodo^hi CD8⁺DC
% of cells
40min
2hr
6hr
CD8⁻DC
OVA-pH rodo^hi CD8⁻DC
G
CD8⁺ DCs
MFI (H-2Kb-SIINFEKL)
16 μg/mL
50 μg/mL
150 μg/mL
CD8⁻ DCs

Figure 6

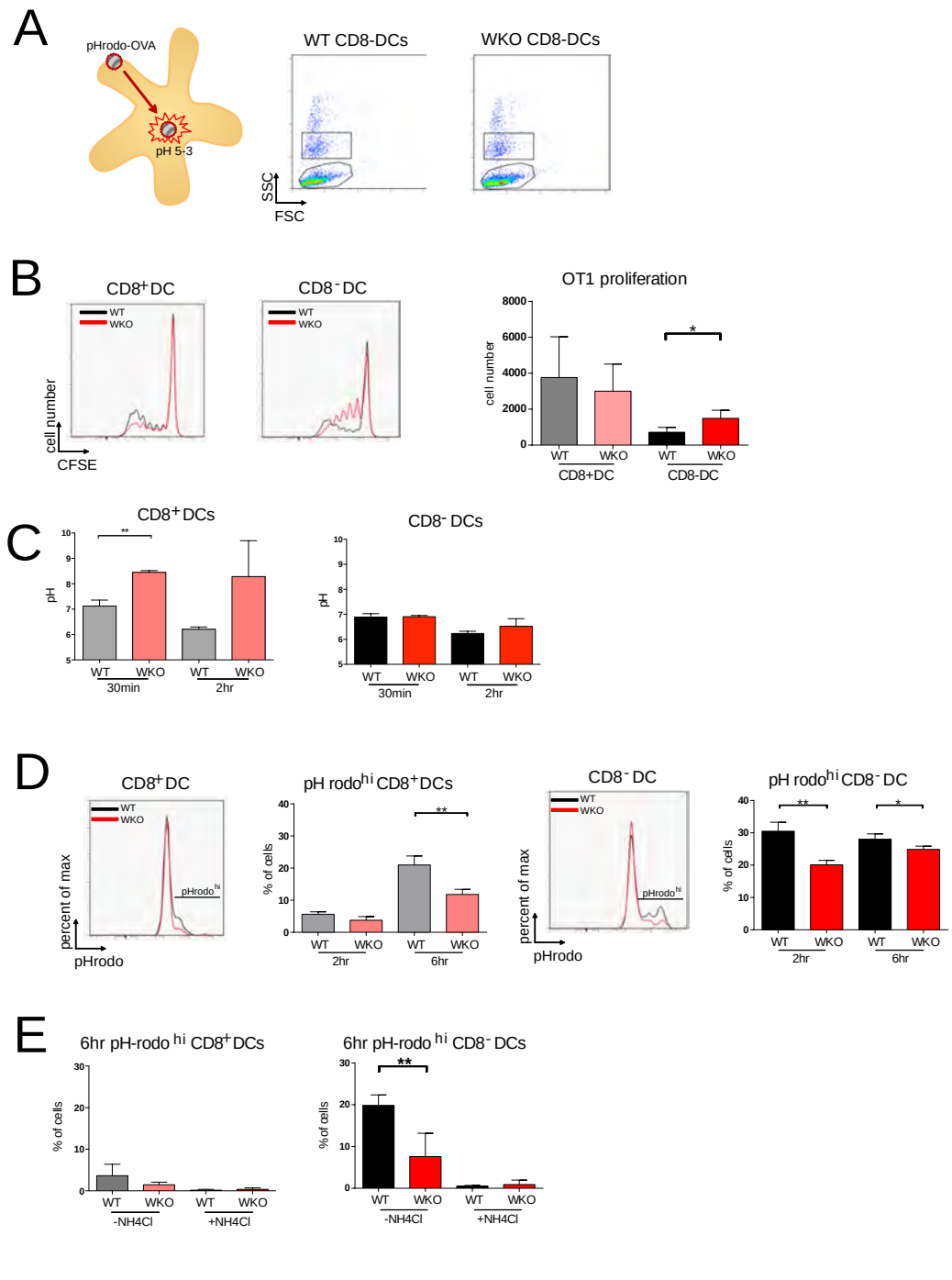

A
pHrodo-OVA
pH 5-3
WT CD8-DCs
WKO CD8-DCs
SSC
FSC
B
CD8+DC
CD8- DC
WT
WKO
cell number
CFSE
OT1 proliferation
8000
6000
4000
2000
0
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
C
CD8+ DCs
CD8- DCs
pH
**
WT
WKO
WT
WKO
30min
2hr
D
CD8+DC
pH rodohi CD8+DCs
CD8- DC
pH rodohi CD8- DC
percent of max
pHrodohi
pHrodo
% of cells
**
*
2hr
6hr
E
6hr pH-rodo hi CD8+DCs
6hr pH-rodo hi CD8- DCs
% of cells
-NH4Cl
+NH4Cl

Figure 7

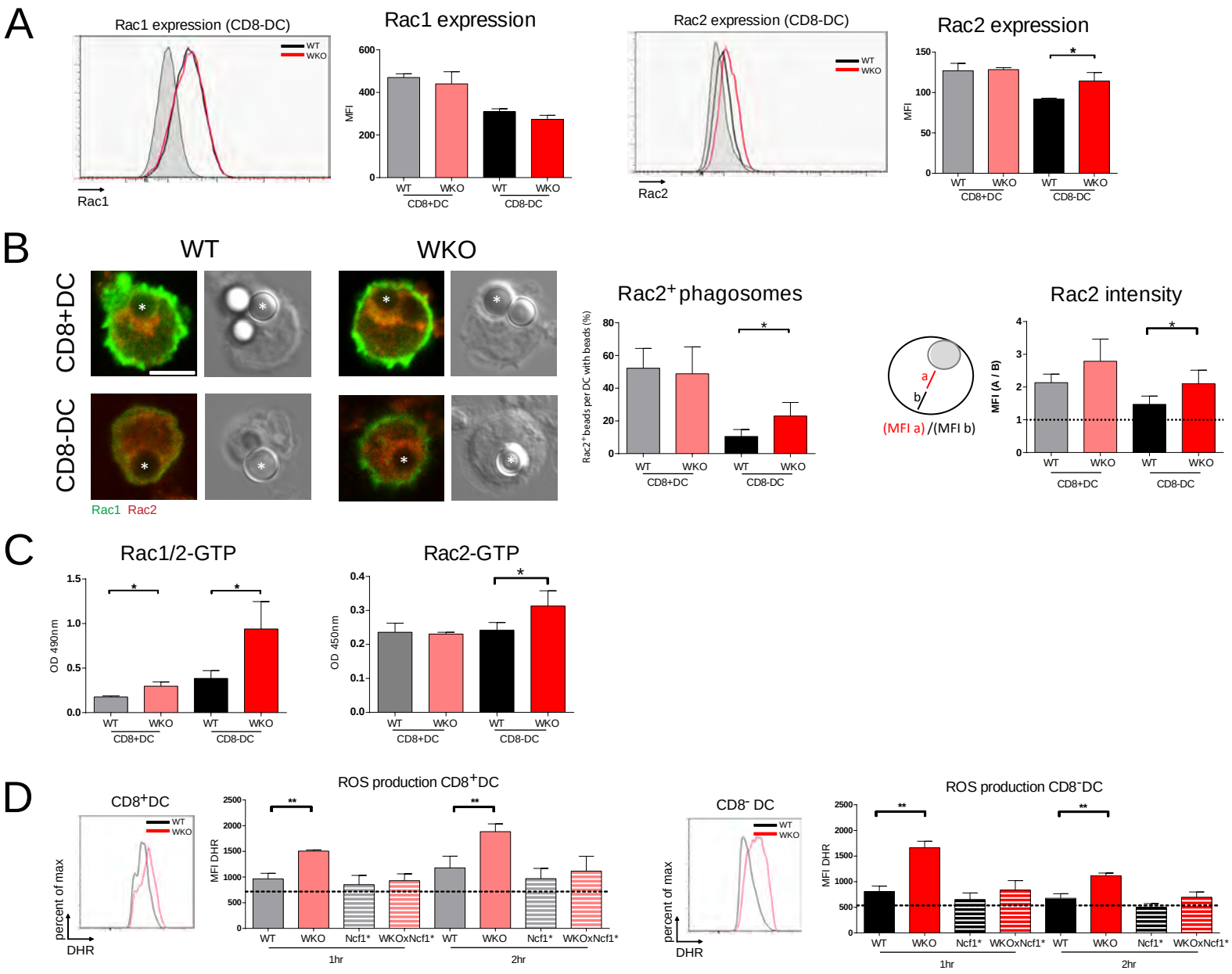
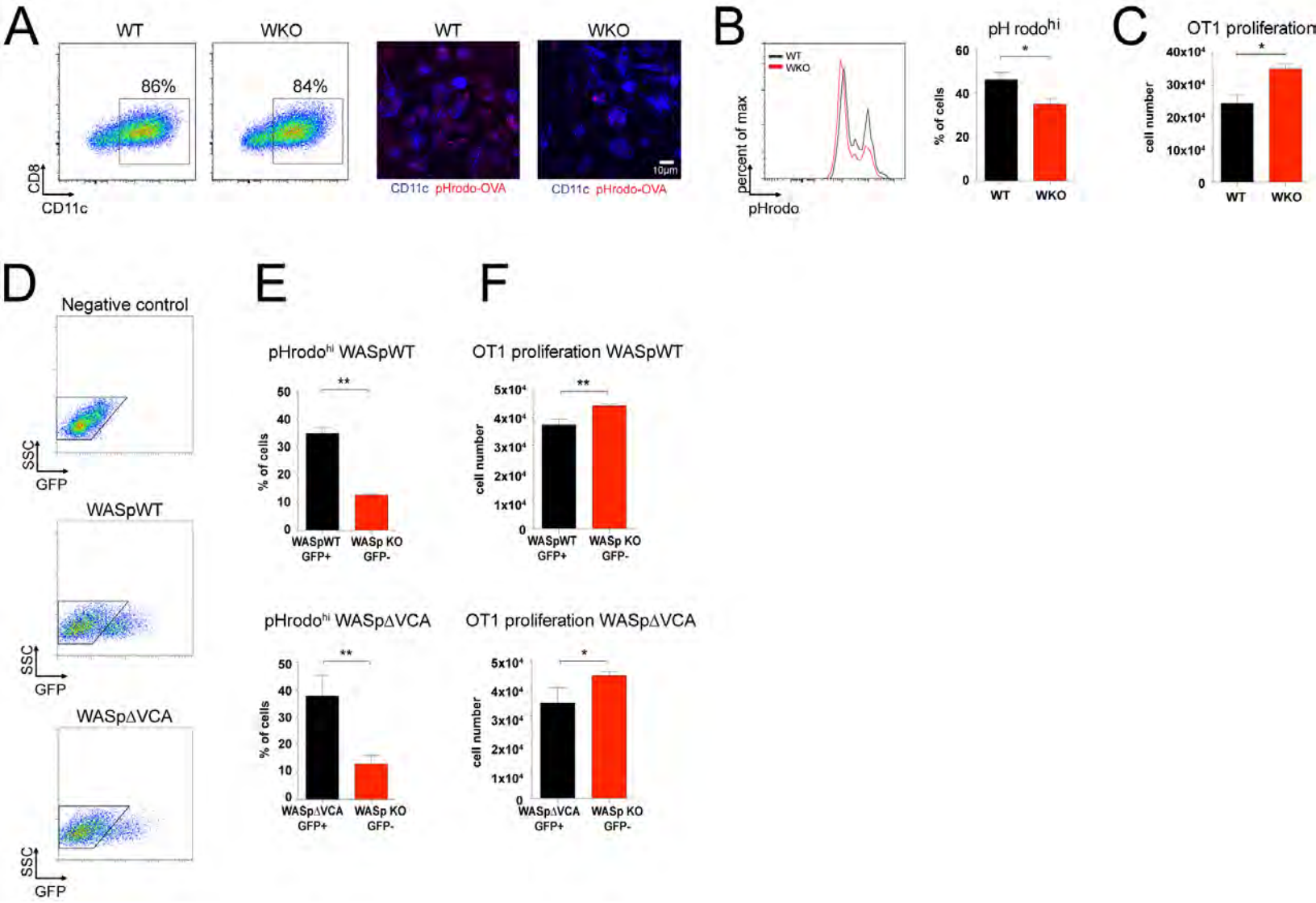

A
Rac1 expression (CD8-DC)
WT
WKO
Rac1
Rac1 expression
MFI
600
400
200
0
WT
WKO
WT
WKO
CD8+DC
CD8-DC
Rac2 expression (CD8-DC)
WT
WKO
Rac2
Rac2 expression
MFI
150
100
50
0
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
B
WT
WKO
CD8+DC
CD8-DC
Rac1 Rac2
Rac2+ phagosomes
Rac2+ beads per DC with beads (%)
80
60
40
20
0
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
a
b
(MFI a) /(MFI b)
Rac2 intensity
MFI (A / B)
4
3
2
1
0
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
C
Rac1/2-GTP
OD 490nm
1.5
1.0
0.5
0.0
*
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
Rac2-GTP
OD 450nm
0.4
0.3
0.2
0.1
0.0
*
WT
WKO
WT
WKO
CD8+DC
CD8-DC
D
CD8+DC
WT
WKO
percent of max
DHR
ROS production CD8+DC
MFI DHR
2500
2000
1500
1000
500
0
**
**
WT
WKO
Ncf1*
WKOxNcf1*
WT
WKO
Ncf1*
WKOxNcf1*
1hr
2hr
CD8- DC
WT
WKO
percent of max
DHR
ROS production CD8-DC
MFI DHR
2500
2000
1500
1000
500
0
**
**
WT
WKO
Ncf1*
WKOxNcf1*
WT
WKO
Ncf1*
WKOxNcf1*
1hr
2hr

Figure 8


A
WT
WKO
86%
84%
CD8
CD11c
WT
WKO
CD11c pHrodo-OVA
CD11c pHrodo-OVA
10µm
B
WT
WKO
percent of max
pHrodo
pH rodo^hi
% of cells
WT
WKO
C
OT1 proliferation
cell number
WT
WKO
D
Negative control
SSC
GFP
WASpWT
SSC
GFP
WASpΔVCA
SSC
GFP
E
pHrodo^hi WASpWT
% of cells
WASpWT GFP+
WASp KO GFP-
pHrodo^hi WASpΔVCA
% of cells
WASpΔVCA GFP+
WASp KO GFP-
F
OT1 proliferation WASpWT
cell number
WASpWT GFP+
WASp KO GFP-
OT1 proliferation WASpΔVCA
cell number
WASpΔVCA GFP+
WASp KO GFP-