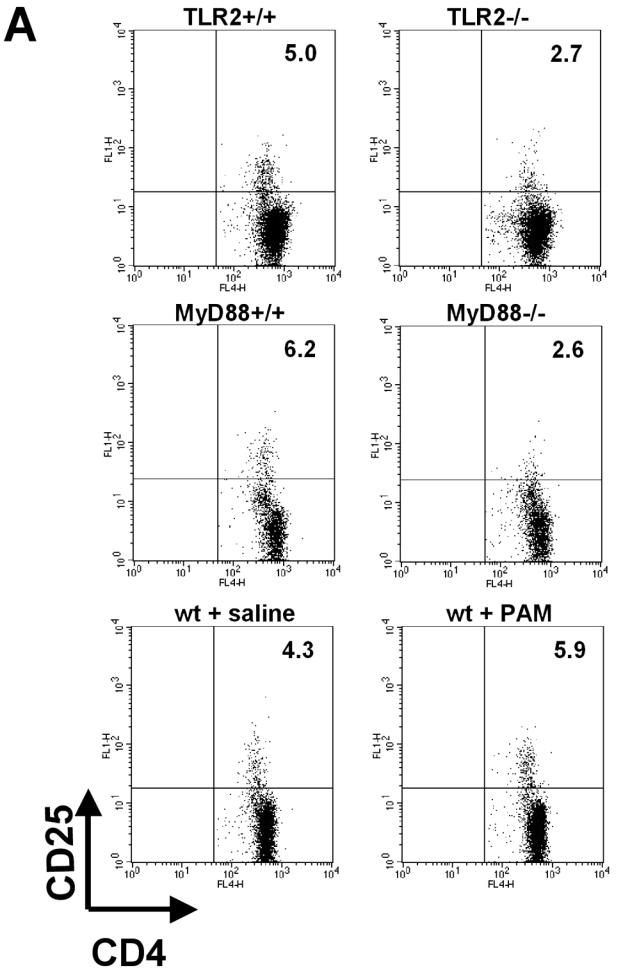
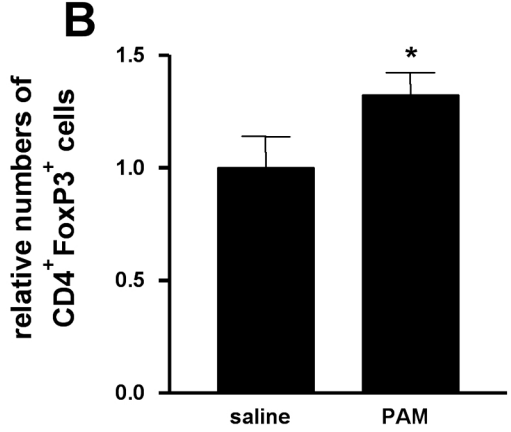
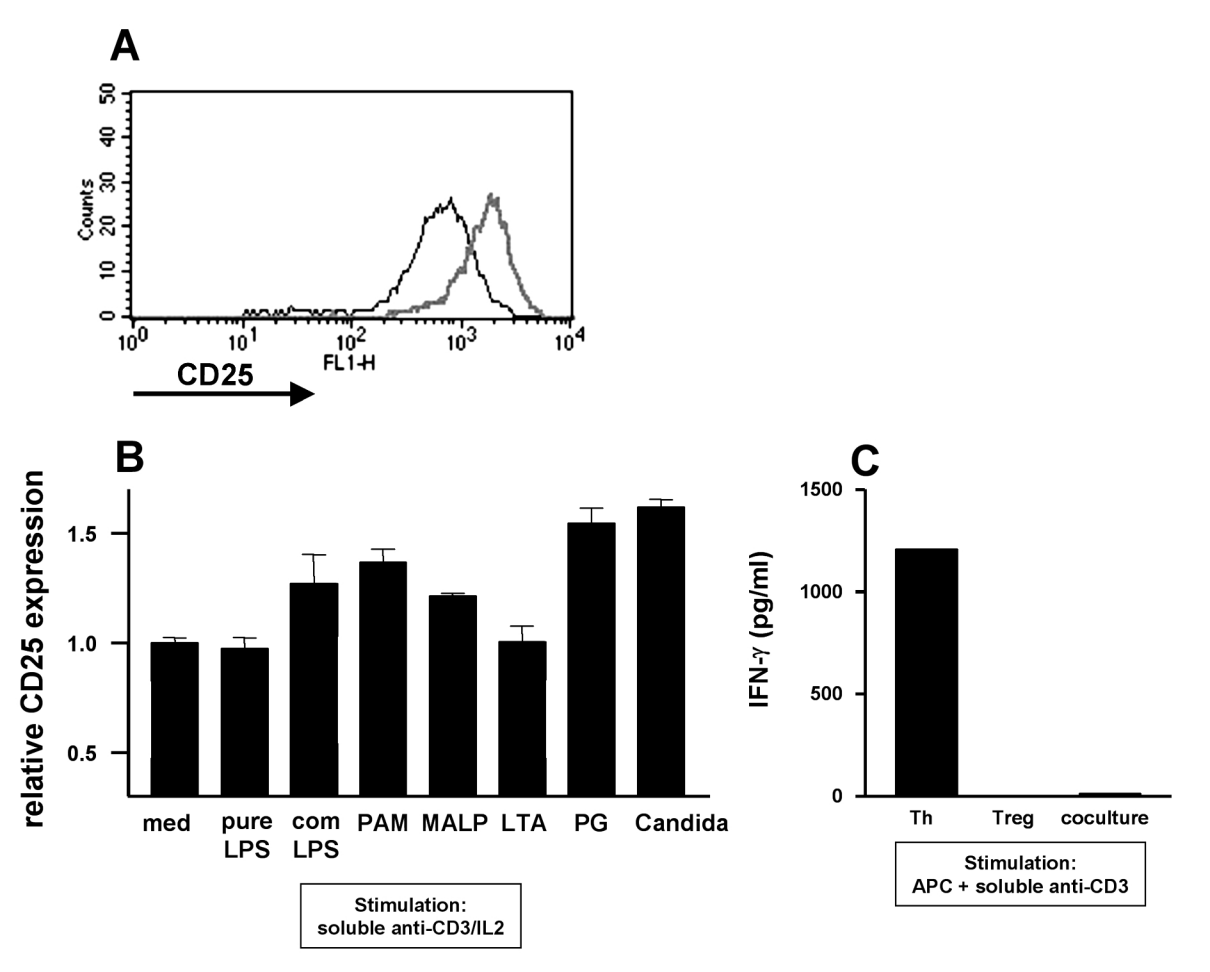
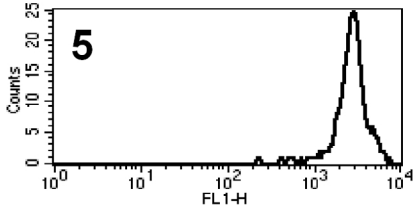

A
TLR2+/+
5.0
TLR2-/-
2.7
MyD88+/+
6.2
MyD88-/-
2.6
wt + saline
4.3
wt + PAM
5.9
FL1-H
FL4-H
CD25
CD4

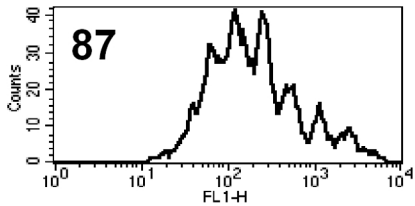

B
relative numbers of CD4+FoxP3+ cells
1.5
1.0
0.5
0.0
*
saline
PAM


A
Counts
0
10
20
30
40
50
10^0
10^1
10^2
10^3
10^4
FL1-H
CD25
B
relative CD25 expression
0.5
1.0
1.5
med
pure LPS
com LPS
PAM
MALP
LTA
PG
Candida
Stimulation: soluble anti-CD3/IL2
C
IFN-γ (pg/ml)
0
500
1000
1500
Th
Treg
coculture
Stimulation: APC + soluble anti-CD3

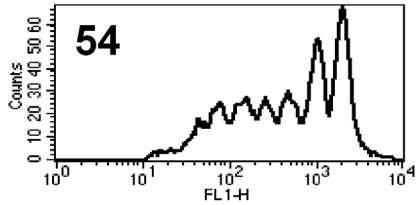
Th

Th

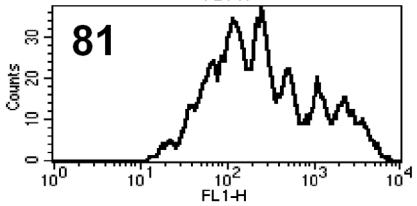




no Treg added



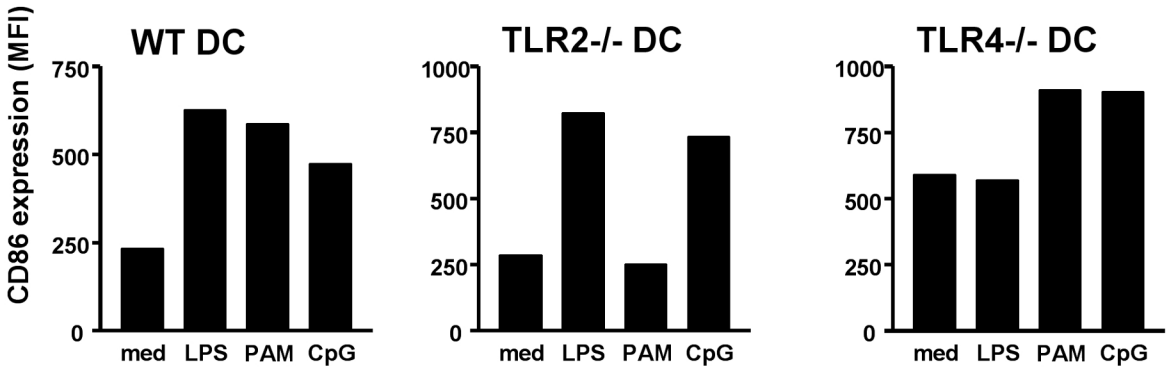
Treg in culture



Treg in transwell

No Stimulation

Stimulation:
Irradiated APC + soluble anti-CD3


CD86 expression (MFI)
WT DC
750
500
250
0
med
LPS
PAM
CpG
TLR2-/- DC
1000
750
500
250
0
med
LPS
PAM
CpG
TLR4-/- DC
1000
750
500
250
0
med
LPS
PAM
CpG

Supplementary figure 1

TLR2-signaling modulates CD4+CD25+ T cell numbers.

(A) Blood from TLR2-/-, MyD88-/- and their wildtype littermate controls as well as from PAM-challenged mice was analyzed by flow cytometry for CD4 and CD25 expressing cells. Values in the upper-right quadrant indicate the percentage $CD4^+CD25^+$ T cells from the total number of $CD4^+$ T cells. Data are representative of three independent experiments with four mice per group

(B) PAM induces an increase in $FoxP3^+$ cells in the periphery. Mice (four per group) were challenged i.p. with 20 μg PAM and two days later the percentage of $CD4^+FoxP3^+$ T cell of the total $CD4^+$ T cells in the blood was determined by flowcytometry. Data indicate the average relative increase with saline control set at 1. (*= $P < 0.05$ for saline control compared with PAM treated group)

Supplementary figure 2

(A) Example of CD25 expression by freshly isolated regulatory T cells incubated for three days in medium with anti-CD3/IL-2 (thin line) or supplemented with TLR2 ligand PAM (thick gray line). **(B)** Effect of different TLR ligands on Treg activation. PAM-expanded Treg cells were cultured with anti-CD3, IL2 and the indicated TLR ligand (10 μg/ml purified LPS, 10 μg/ml commercial LPS, 2 μg/ml PAM, 2μg/ml MALP-2, 10μg/ml LTA, 10μg/ml Peptidoglycan (PG), and 2 $x10^5$ heat-killed *Candida*/ml). The cells were incubated for three days and subsequently CD25 expression was analyzed by flow cytometry. Representative results from two experiments are shown. **(C)** In vitro suppression assay: PAM-expanded Treg were rested for at least six days in the absence of TLR-ligands and subsequently cocultured for three days with 10^4 fresh naïve $CD4^+$ T-cells, irradiated APC and anti-CD3. After three days, IFN-γ production was measured in the supernatant using the murine inflammation CBA kit.

Supplementary figure 3

PAM-Treg suppression is cell-contact dependent. Transwell suppression assay: 10^6 Freshly isolated CFSE labeled $CD4^+$ Th cells were incubated with 10^6 irradiated APC and 1μg/ml anti-CD3 per well of a 24-wells plate. If indicated 10^6 PAM expanded Treg and 10^6 irradiated APC were added in the culture with the fresh Th cells or in the upper-chamber of the transwell (pore size 0.4μm). After four days of culture, CFSE fluorescence was measured by flowcytometry. The value indicates the percentage of cells in the proliferative fraction.

Supplementary figure 4

TLR-ligand induced activation of mouse bone marrow derived DC of wildtype, TLR2-/- and TLR4-/- origin. Day 7 bone marrow derived DC were incubated for 24 hours with the indicated TLR ligands and subsequently the expression of activation marker CD86 was measured by flow cytometry. A representative result from two experiments is shown.