Figure legends

Figure 1:

Co-cultured NK cells and DCs in the presence of VLPs induce maturation markers on DCs. Box and whisker plots of MFI ratio (MFI of experimental conditions divided by MFI of DCs alone) in presence (in grey) or absence of cell-cell contact (transwell condition, in white). (A) MFI ratio of CD86, (B) MFI ratio of HLA-DR, (C) MFI ratio of CD80, (D) MFI ratio of CD83. (E) CD40 blockade (in black) inhibits the upregulation of HLA-DR on DC in the presence of NK cells and VLPs, n= 4-9 . *p<0,05, **p<0,005 , ***p<0,001 Mann-Whitney test.

Figure 2:

NKp30 ligands are down-regulated on DC in the presence of NK cells and VLP. NKp30 ligand expression on DCs was assessed by flow cytometry using soluble recombinant NKp30-Ig fusion proteins. (A) MFI ratio of NKp30-Ig. Scatter plots are shown for the different conditions (DC irrelevant= negative controls: irrelevant–higG1-Fc fusion protein staining) n= 3-6. *p<0,05, **p<0,005, Mann-Whitney test. (B) Representative NKp30-Ig. staining on DC alone (black), in the presence of NK and VLPs (grey) and secondary antibody staining without NKp30-Ig (dashed grey line) or + irrelevant : irrelevant–higG1-Fc fusion protein (grey line). (C) Representative NKp30-Ig. staining on K562 alone (black), in the presence of VLPs (grey) and secondary antibody staining without NKp30-Ig (dashed grey line).

Figure 3:

NK cells modulate cytokine secretion of VLP-activated DCs. The levels of (A) IL12p70 and (B) IL-10 in culture supernatants of DCs, DCs in the presence of VLPs, NK cells or NK cells

and VLPs were measured by ELISA (realized in duplicate). Box and whisker plots are shown for the different conditions, n= 4-9. * p<0,05,**p<0,005 Mann-Whitney test.

Figure 4:

VLP-activated DCs further activate NK cells via cell-cell contacts. Box and whisker plots of CD69 and HLA-DR expression in presence (in grey) or absence of cell-cell contact (transwell condition, in white). (A) MFI ratio of CD69 (MFI of experimental conditions divided by MFI of NK cells alone), (B) % HLA-DR on NK cells. (C) MFI ratio of CD69 on $CD56^{bright}$ and $CD56^{dim}$ NK cells, (D) MFI of HLA-DR on $CD56^{bright}$ and $CD56^{dim}$ NK cells. (E-F) IL-12 neutralization (in black) inhibits the upregulation of CD69 (E) and HLA-DR (F) on NK cells in the presence of DCs and VLPs, n=4- 9. *p<0,05, **p<0,005, ***p<0,001 Mann-Whitney test.

Figure 5:

VLP-activated DCs potentiate NK cell effector functions. (A) Co-cultured DCs and NK cells in the presence of VLPs induce production of IFN-γ (ELISA realized in duplicate). (B) Intracellular staining with monensin after 20h showed that NK cells and DCs produce both IFN-γ in the NK-DC-VLP condition, representative figure of 2 experiments. (C) This increased production of IFN-γ is on cell-cell contact dependent (transwell condition, in white) and (D) IL-12 (in black). Box and whisker plots are shown for the different conditions, n= 4-9. *p<0,05, **p<0,005, ***p<0,001 Mann-Whitney test.

Figure 6:

Co-cultured DCs and NK cells in the presence of VLPs induce NK cell cytotoxic activity. (A) Percentage of tumor growth inhibition by NK cells against CasKi cell line in a 7 days MTT

assay in the presence of VLPs, DCs, DCs and VLPs shown as scatter plot, n=3. (B) NK cell cytotoxic activity against CasKi cell line in a 4h ^{51}Cr release assay (realized in triplicate) in the presence of DCs, DCs and lysate of insect cells infected with WT baculovirus, VLPs, DCs and VLPs. n= 7-9, *p<0,05, Mann-Whitney test.

Figure 7:

DC-NK crosstalk in the presence of VLPs. VLPs induce upregulation of CD86, HLA-DR, CD80 and CD83 on imDCs and secretion of IL-10 and IL-12. The secretion of IL-12 stimulates NK cells (upregulation of CD69 and HLA-DR) and NK cells secrete higher amounts of IFN-γ, which could help adaptive T cell immunity. These activated NK cells become highly cytotoxic against HPV^+ cells, which result in a release of apoptotic bodies, a source of Ag for adaptive immunity. We observed a further upregulation of CD86 and HLA-DR on DCs and an increase of the IL-12p70 secretion, but no increase of the production of the immunosuppressive cytokine IL-10. Besides IL-12, CD40 is also implicated in this crosstalk.

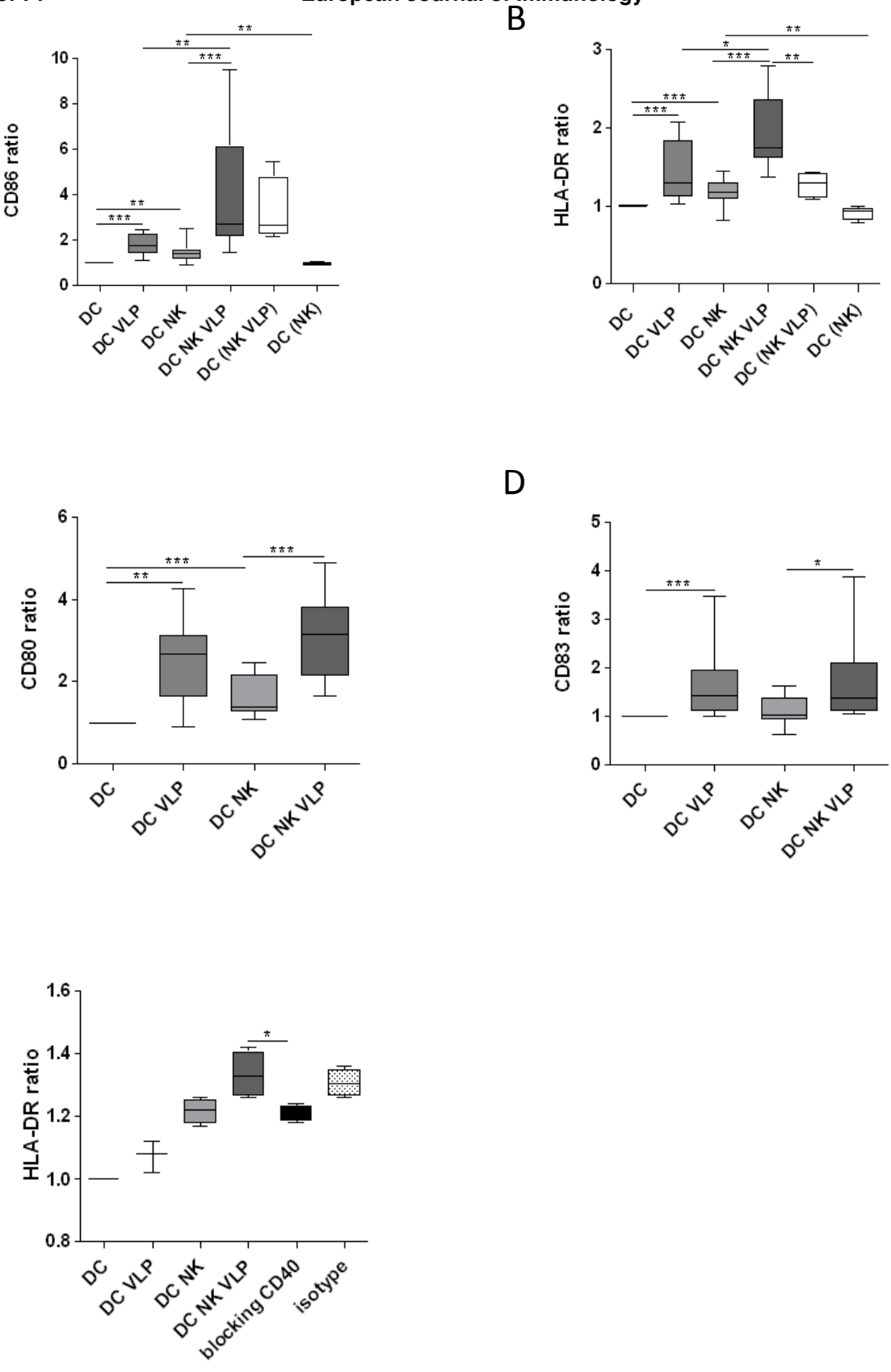

A
CD86 ratio
DC
DC VLP
DC NK
DC NK VLP
DC (NK VLP)
DC (NK)
B
HLA-DR ratio
DC
DC VLP
DC NK
DC NK VLP
DC (NK VLP)
DC (NK)
C
CD80 ratio
DC
DC VLP
DC NK
DC NK VLP
D
CD83 ratio
DC
DC VLP
DC NK
DC NK VLP
E
HLA-DR ratio
DC
DC VLP
DC NK
DC NK VLP
blocking CD40
isotype

Figure 1

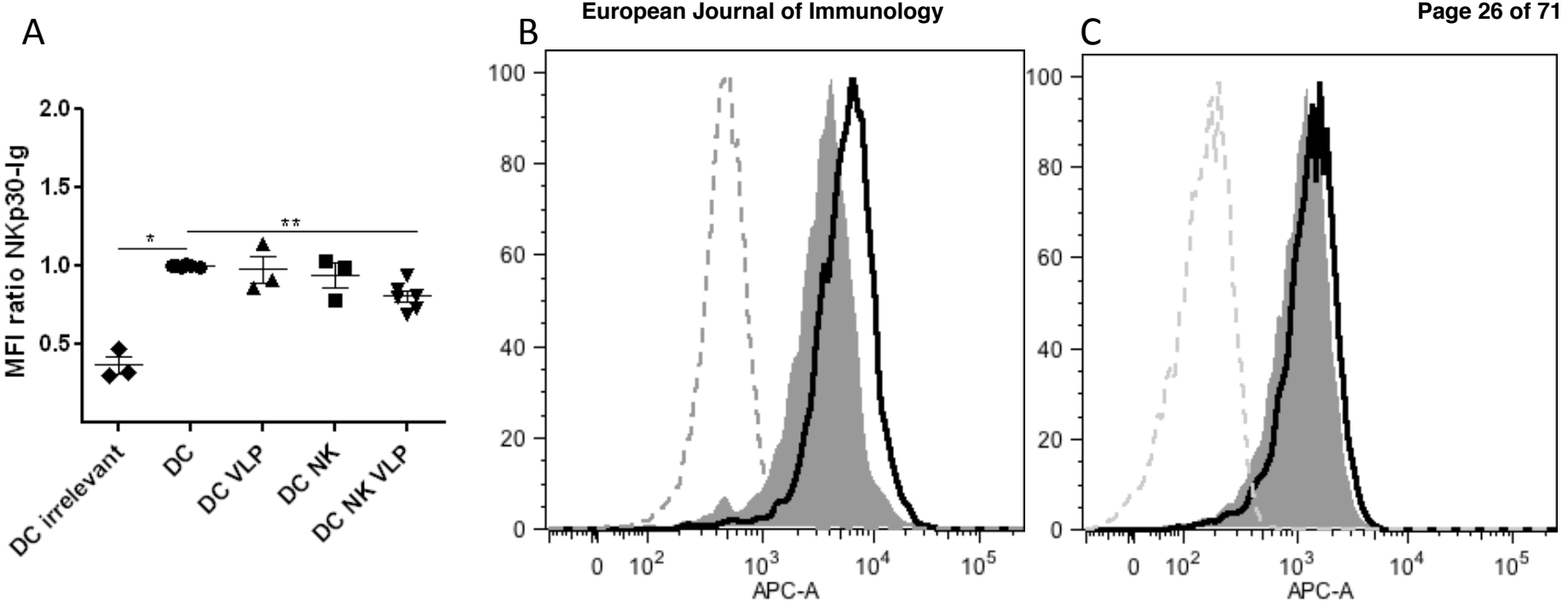
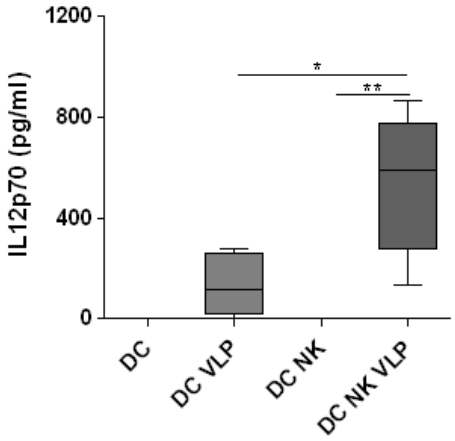

A
B
C
MFI ratio NKp30-Ig
2.0
1.5
1.0
0.5
*
**
DC irrelevant
DC
DC VLP
DC NK
DC NK VLP
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5
APC-A

Figure 2

A


IL12p70 (pg/ml)
1200
800
400
0
*
**
DC
DC VLP
DC NK
DC NK VLP

B

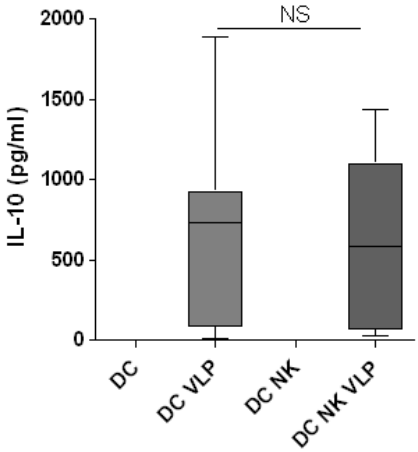

IL-10 (pg/ml)
2000
1500
1000
500
0
NS
DC
DC VLP
DC NK
DC NK VLP

Figure 3

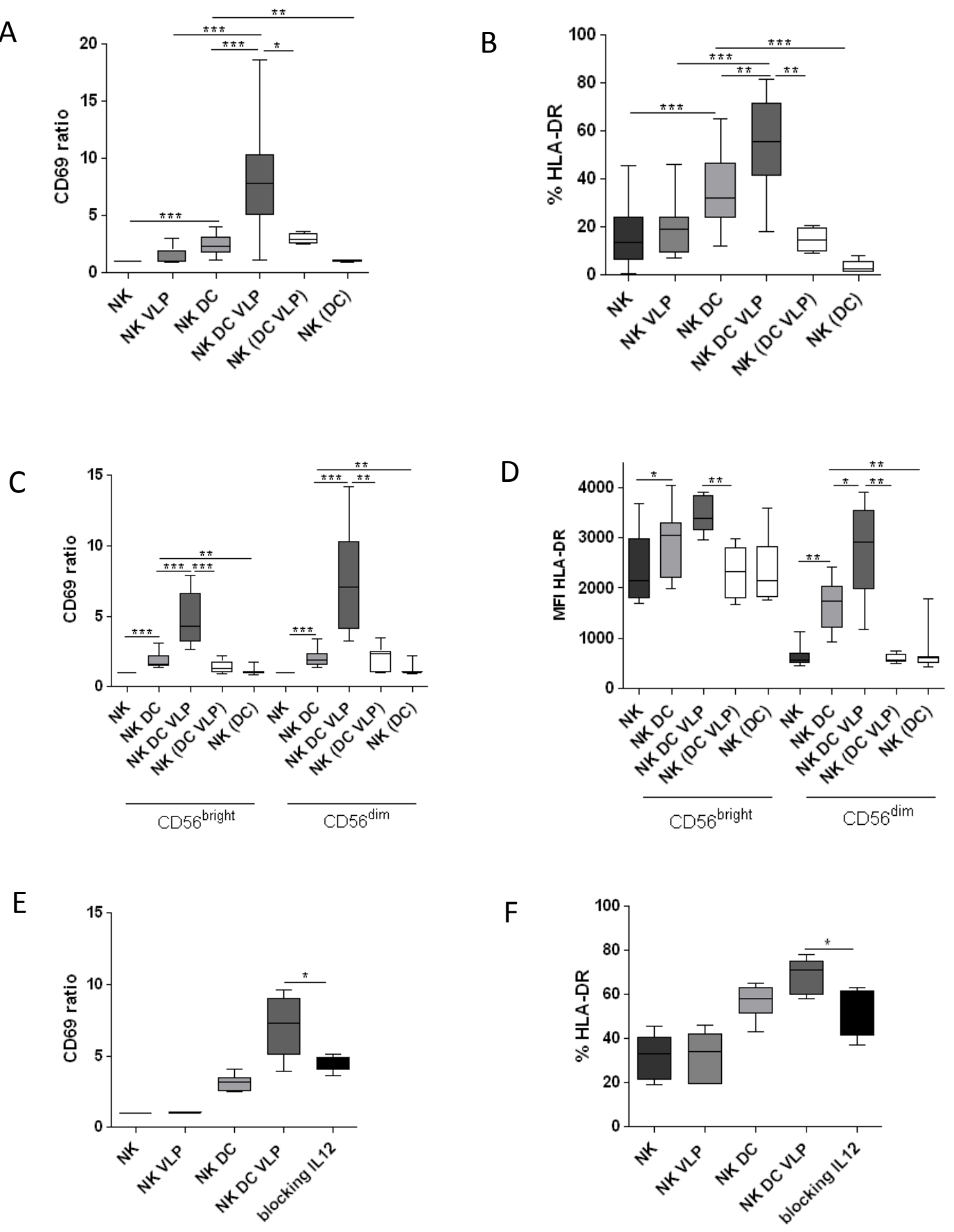

A
CD69 ratio
NK
NK VLP
NK DC
NK DC VLP
NK (DC VLP)
NK (DC)
B
% HLA-DR
C
CD69 ratio
CD56bright
CD56dim
D
MFI HLA-DR
E
CD69 ratio
blocking IL12
F
% HLA-DR

Figure 4

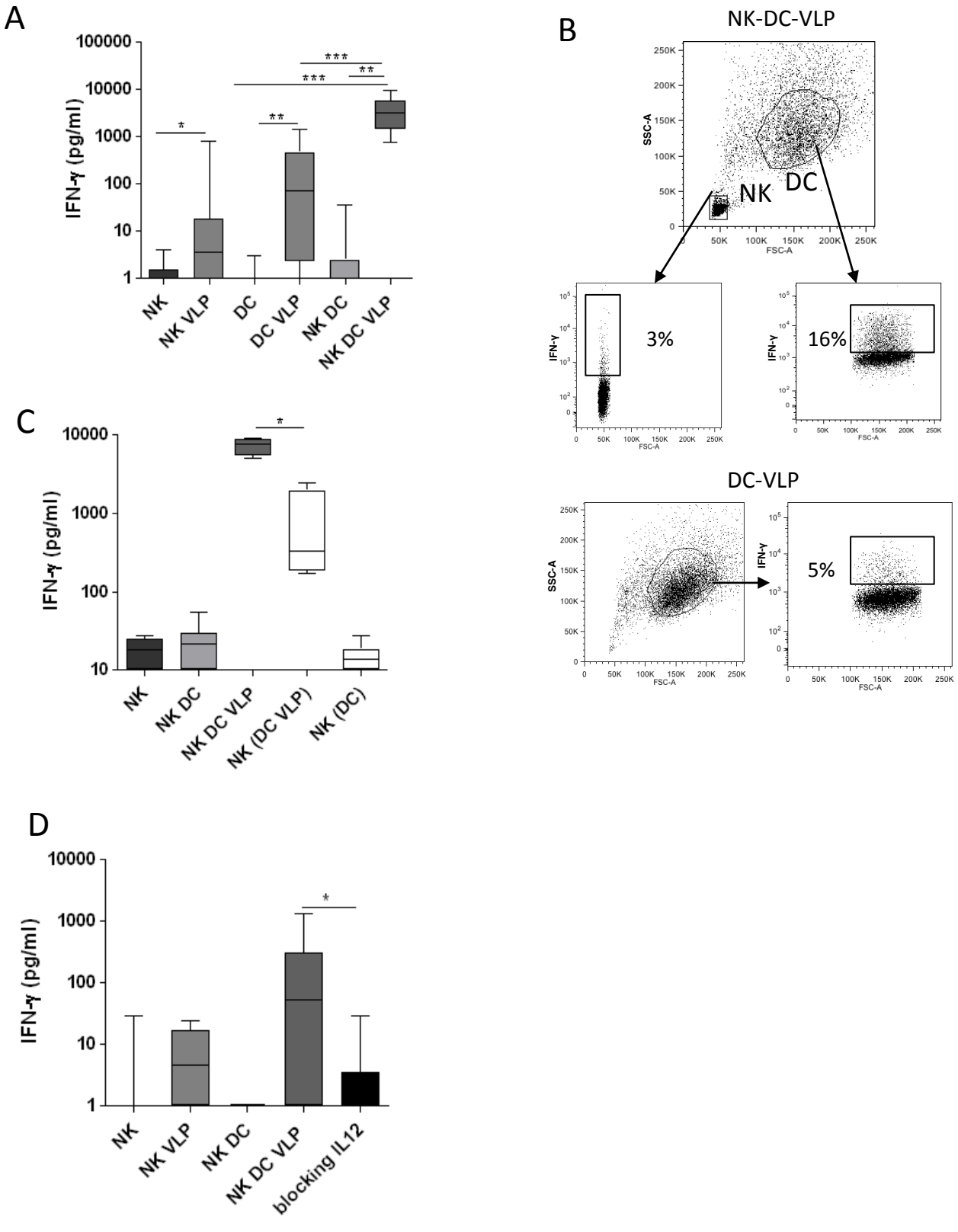

A
IFN-γ (pg/ml)
100000
10000
1000
100
10
1
*
**
***
***
**
NK
NK VLP
DC
DC VLP
NK DC
NK DC VLP
B
NK-DC-VLP
SSC-A
FSC-A
NK
DC
IFN-γ
3%
16%
DC-VLP
5%
C
IFN-γ (pg/ml)
10000
1000
100
10
*
NK
NK DC
NK DC VLP
NK (DC VLP)
NK (DC)
D
IFN-γ (pg/ml)
10000
1000
100
10
1
*
NK
NK VLP
NK DC
NK DC VLP
blocking IL12

Figure 5

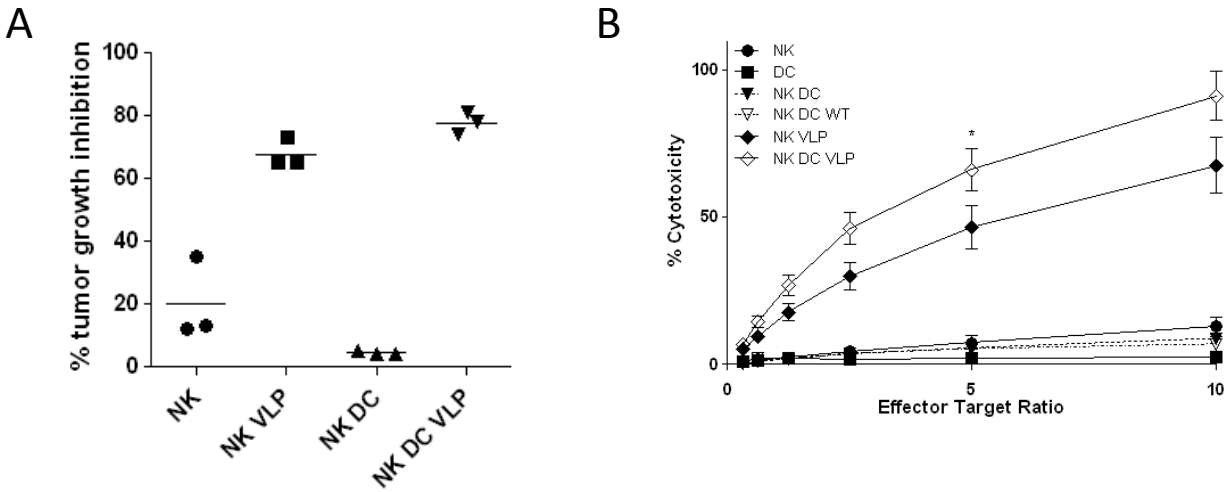

A
% tumor growth inhibition
100
80
60
40
20
0
NK
NK VLP
NK DC
NK DC VLP
B
% Cytotoxicity
100
50
0
0
5
10
Effector Target Ratio
*
NK
DC
NK DC
NK DC WT
NK VLP
NK DC VLP

Figure 6

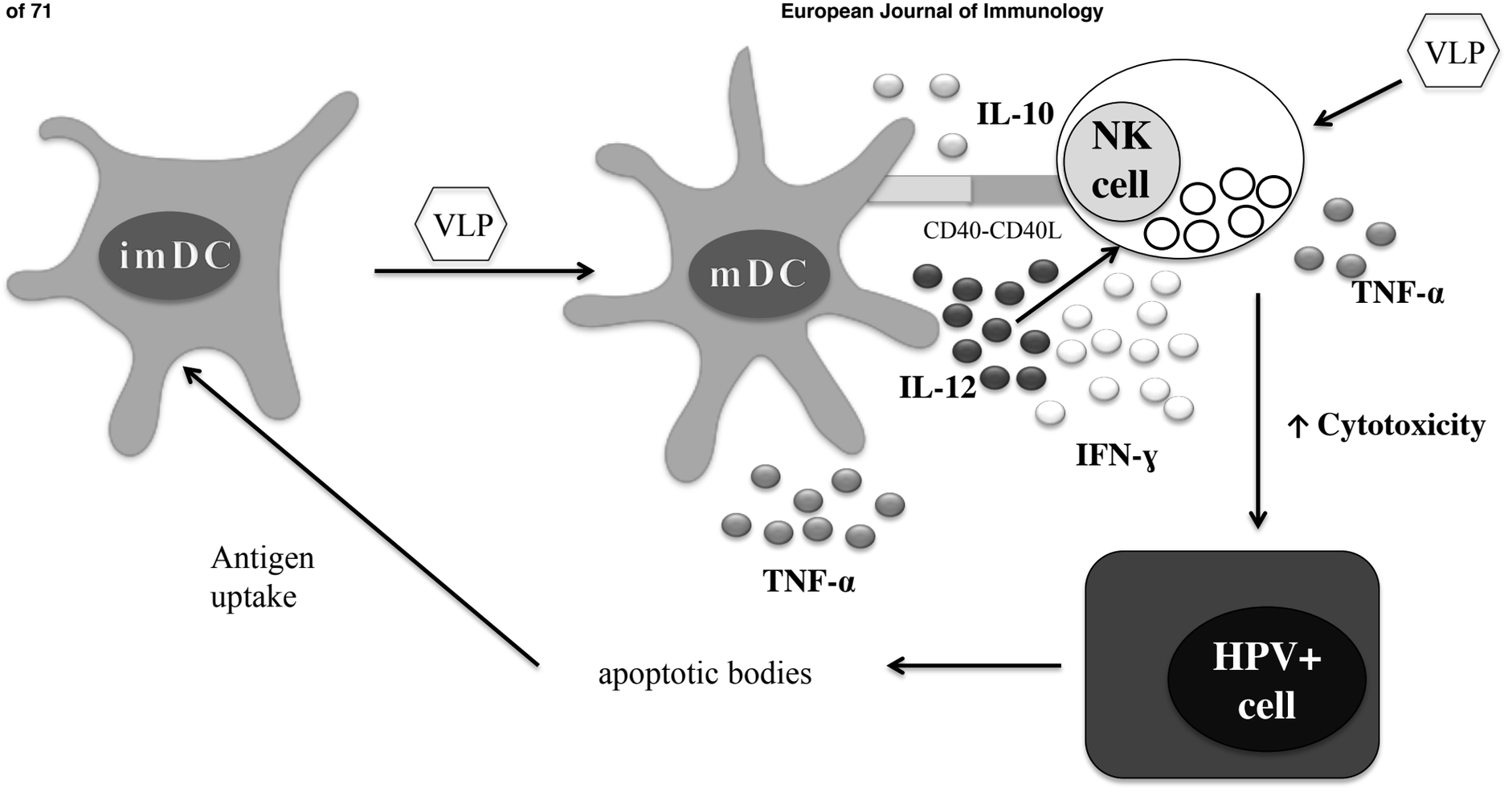

VLP
IL-10
NK cell
imDC
VLP
mDC
CD40-CD40L
TNF-α
IL-12
IFN-ɣ
↑ Cytotoxicity
TNF-α
Antigen uptake
apoptotic bodies
HPV+ cell

Figure 7