Figure S1. AdV transduction does not sensitize DC to NK cell-mediated killing
iDC, Ad.LacZ (500 MOI) or mDC were labeled with CTO, mixed with resting NK cells in the indicated E:T (NK cell:DC) ratios, and tested 24h after treatment for their susceptibility to NK cell killing using (A) CTO-based cytotoxicity or (B) granzyme B ELISPOT assays. In (A), percent of specific cell death is shown, based on the percent of $CTO^{+}7\text{-}AAD^{+}$ DC according to FACS analysis of 10,000 DC. In (B), ELISPOT assays were performed in triplicates, and data are means + SEM. Data are representative of three experiments.

Figure S2. AdV transduction enhances DC maturation in a dose-dependent manner, and DC maturation is further enhanced by NK cell presence
DC alone or together with NK cells were cultured as described in Fig. 2, and tested for cell surface phenotypic marker expression using multicolor flow cytometry. Bars represent the coefficient of variation for 5,000 events acquired. Data are representative of ten experiments performed.

Figure S3. Ad,DC and mDC upregulate NKp30 expression and tumor-specific granzyme B secretion by NK cells
NK cells and DC co-cultures were performed as described in Fig. 1C–E. (A) Expression of NKG2D and (B) NKp30 activating receptors on NK cells was measured after their co-culture with DC by FACS analysis. MFI values are presented. (C) NK cells from DC/NK cell co-cultures were tested for the ability to secrete granzyme B in response to K562 tumor cell targets (5 effectors per 1 target) using ELISPOT assays. Spontaneous granzyme B secretion in tumor-free media was also measured. ELISPOT assays were performed in triplicates, and data are means + SEM. Data are representative of three experiments performed.

Figure S4. Growth of individual mouse tumors
Mice were injected with PBS into tumor (Vehicle), NK cells alone into tumor (NK), Ad.DC alone into tumor, mixture of iDC and NK (iDC/NK) into tumor, Ad.DC into contra lateral, tumor-free flank and NK cells into tumor (Ad.DCcl-NKt), and mixture of Ad.DC and NK (Ad.DC/NK) into tumor. Tumor growth was followed as explained in Fig. 3.

Figure S5. Percent inhibition of tumor growth in Ad.DC/NK-treated mice compared to control mice

Figure S6. Statistical significance differences of tumor volumes of Ad.DC/NK-treated mice compared to control mice

Figure S7. Adherent DC strongly co-express tmTNF and *trans*-IL-15 on small restricted areas of dendrites at sites of cell-to-cell contacts
Topography of tmTNF and *trans*-IL-15 expression on adherent iDC, Ad.DC, and mDC was examined with confocal microscopy. Reconstructed images of seven 0.4μm thick optical sections are shown. Results are representative of three experiments.

Figure S8. **IL-15Rα expression is increased on Ad.DC and mDC**
IL-15Rα expression levels by iDC, Ad.DC, and mDC prior to their co-culture with NK cells were analyzed by FACS.

Figure S9. Ad.DC and mDC have different secretion profiles for solTNF and solIL-15
Cell-free supernatants of cultured iDC, Ad.DC, and mDC were tested at different time points for soluble TNF (top panel) and IL-15 (bottom panel) by ELISA and cytokine multiplex assays, respectively. To control for any potential cell debris contributions to these results, Brefeldin A was used as a secretion blocker. Data are representative of three experiments.

Figure S10. NK cell activation by Ad.DC is induced via TNF ligation of TNFR1 and TNFR2
(A) Ad.DC and (B) mDC co-cultures with NK cells and measurements of NK cell activation were performed as described in Fig. 5. DC and NK cells were co-cultured for 24h in the presence of 20μg/ml of control human and mouse IgG (IgG), anti-TNF antibody infliximab (αTNF), anti-TNFR1 (αR1) or anti-TNFR2 (αR2) antibodies. Data are means + SEM of triplicates, and are representative of five experiments. ***** - $p < 0.05$.

Figure S11. Soluble factors secreted by mDC have a low ability to activate NK cells, and this ability is independent of TNF or IL-15
mDC supernatants were co-cultured with NK cells for 24h in the presence of 20μg/ml of human and mouse nonreactive IgG (IgG), anti-TNF antibody (αTNF), TNFR2-Fc construct (EN), anti-IL-15 antibody (αIL-15), or both αTNF and αIL-15 (αTNF + αIL-15). NK cell activation was tested by IFN-γ ELISPOT. Data are representative of three experiments and represent means + SEM.

Figure S1

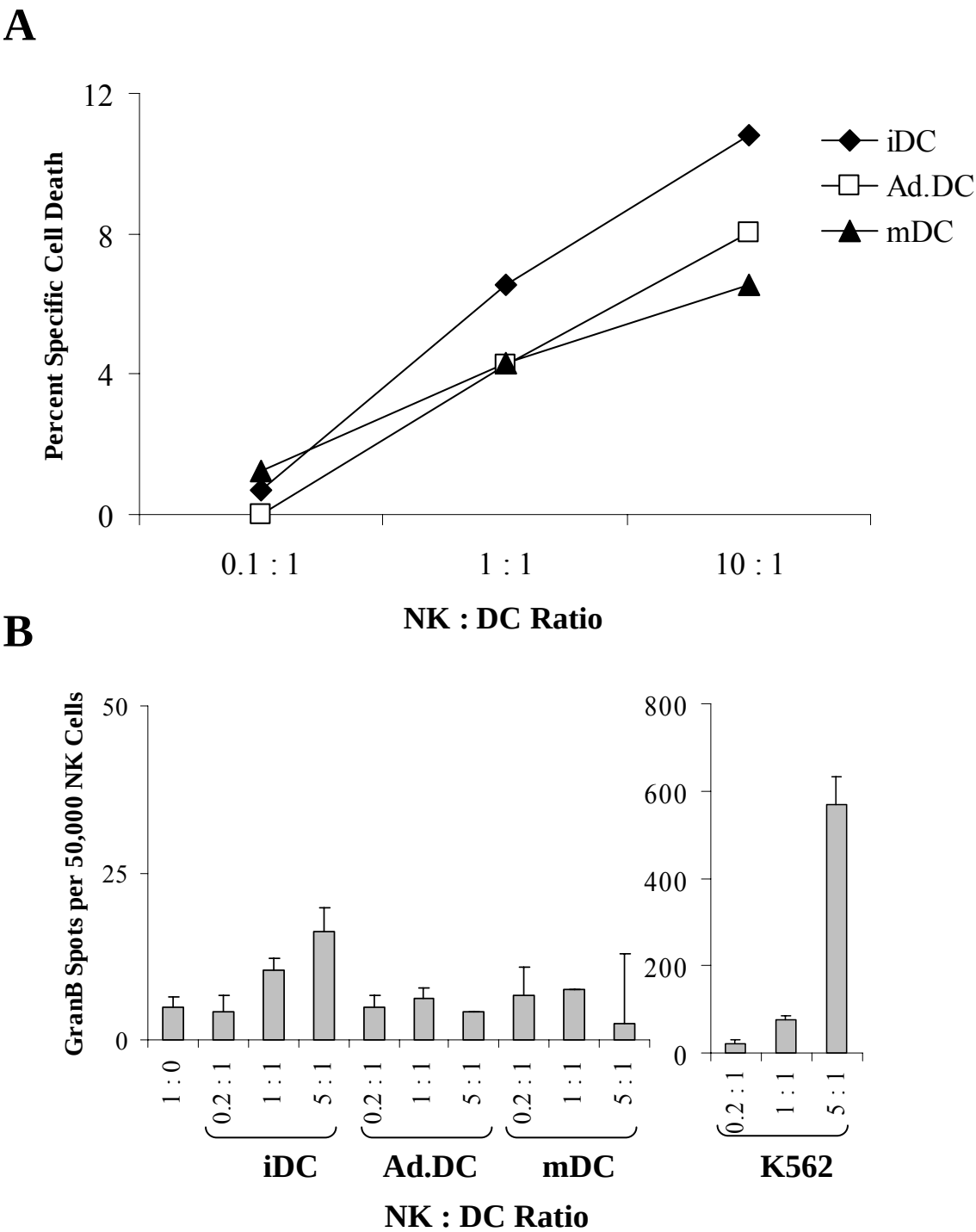

A
12
8
4
0
Percent Specific Cell Death
iDC
Ad.DC
mDC
0.1 : 1
1 : 1
10 : 1
NK : DC Ratio
B
50
25
0
800
600
400
200
0
GranB Spots per 50,000 NK Cells
1 : 0
0.2 : 1
1 : 1
5 : 1
iDC
Ad.DC
mDC
K562
NK : DC Ratio

Figure S2

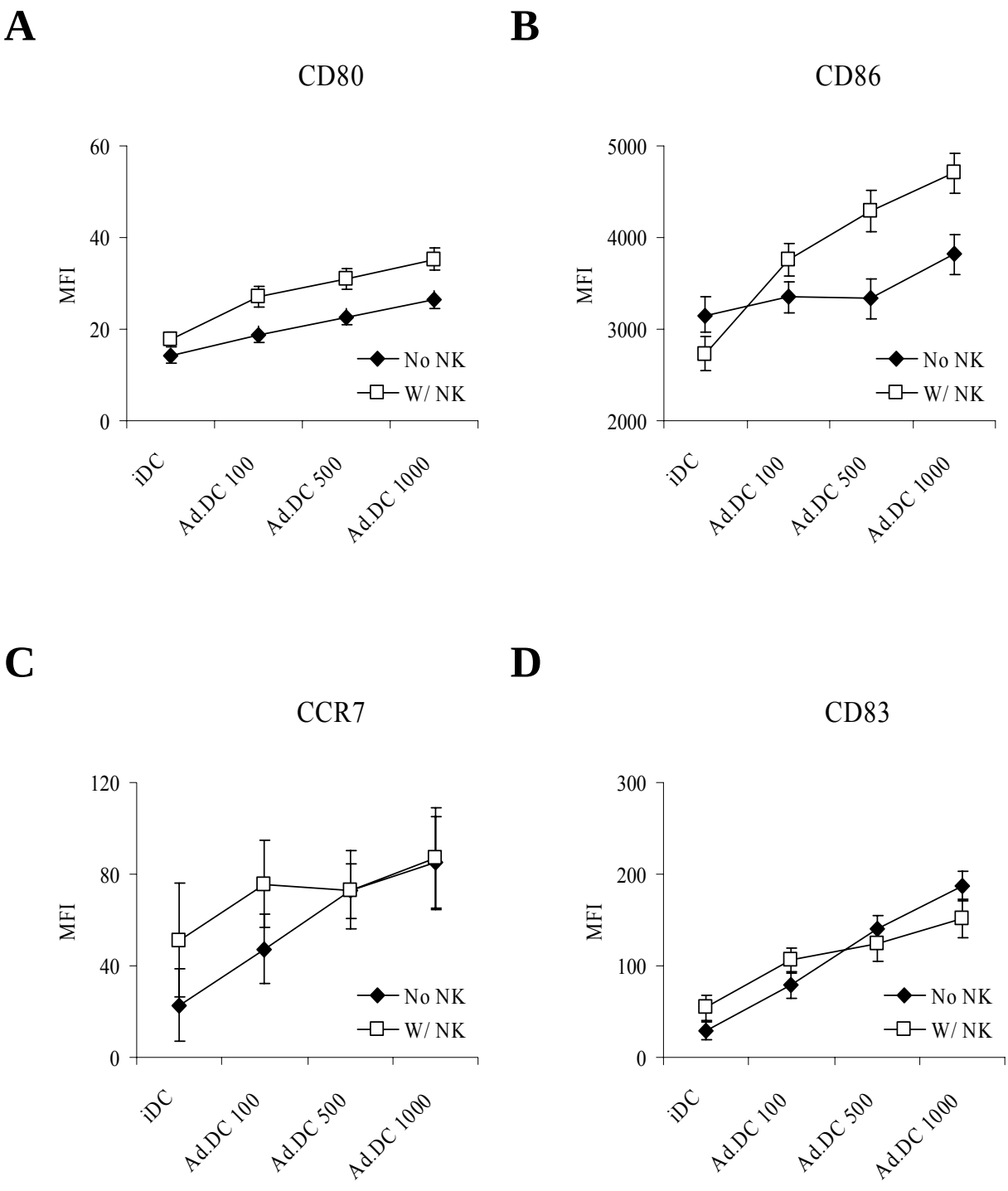

A
CD80
MFI
60
40
20
0
No NK
W/ NK
iDC
Ad.DC 100
Ad.DC 500
Ad.DC 1000
B
CD86
MFI
5000
4000
3000
2000
No NK
W/ NK
iDC
Ad.DC 100
Ad.DC 500
Ad.DC 1000
C
CCR7
MFI
120
80
40
0
No NK
W/ NK
iDC
Ad.DC 100
Ad.DC 500
Ad.DC 1000
D
CD83
MFI
300
200
100
0
No NK
W/ NK
iDC
Ad.DC 100
Ad.DC 500
Ad.DC 1000

Figure S3

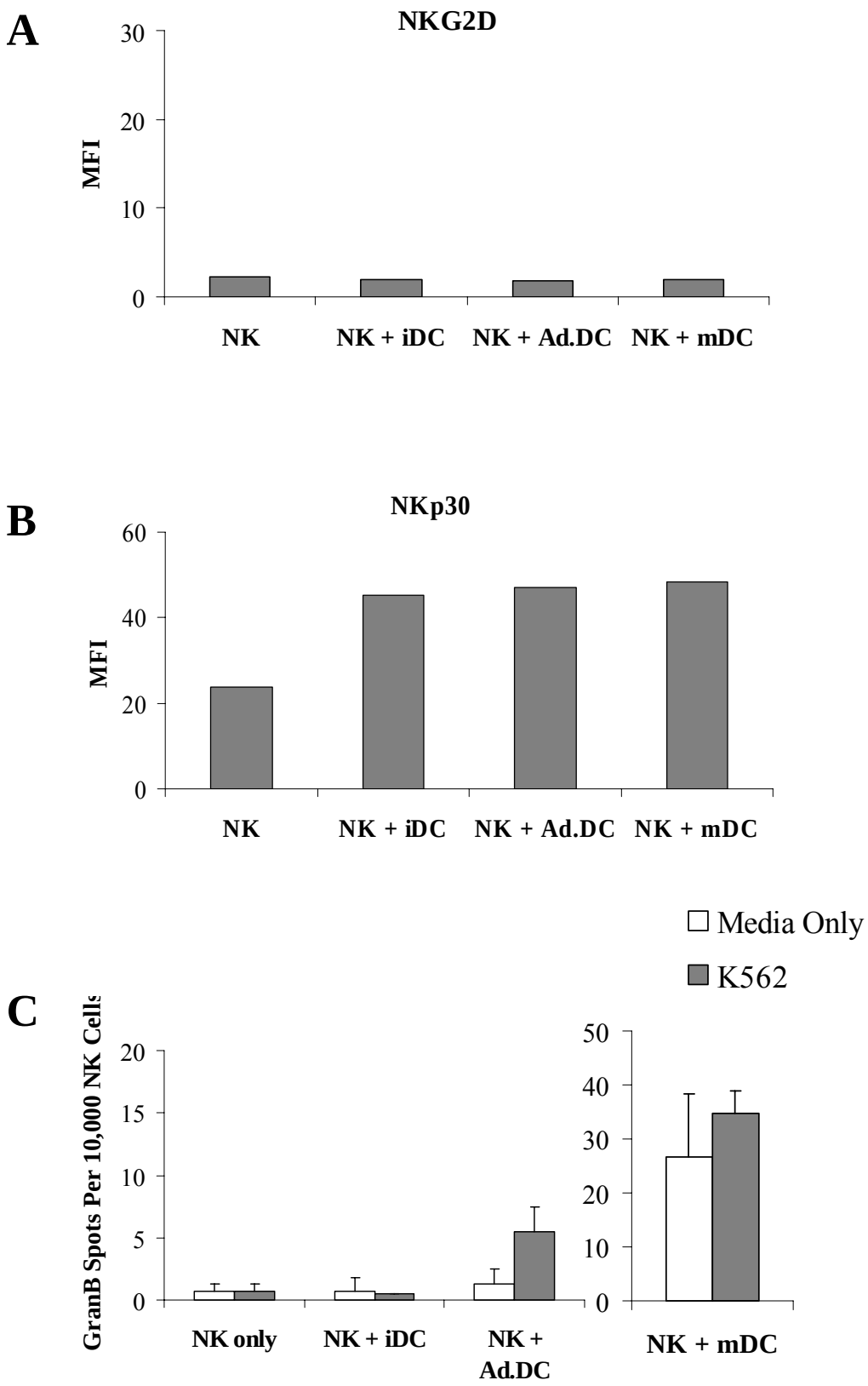

A
NKG2D
MFI
30
20
10
0
NK
NK + iDC
NK + Ad.DC
NK + mDC
B
NKp30
MFI
60
40
20
0
NK
NK + iDC
NK + Ad.DC
NK + mDC
C
Media Only
K562
GranB Spots Per 10,000 NK Cells
20
15
10
5
0
50
40
30
20
10
0
NK only
NK + iDC
NK + Ad.DC
NK + mDC

Figure S4

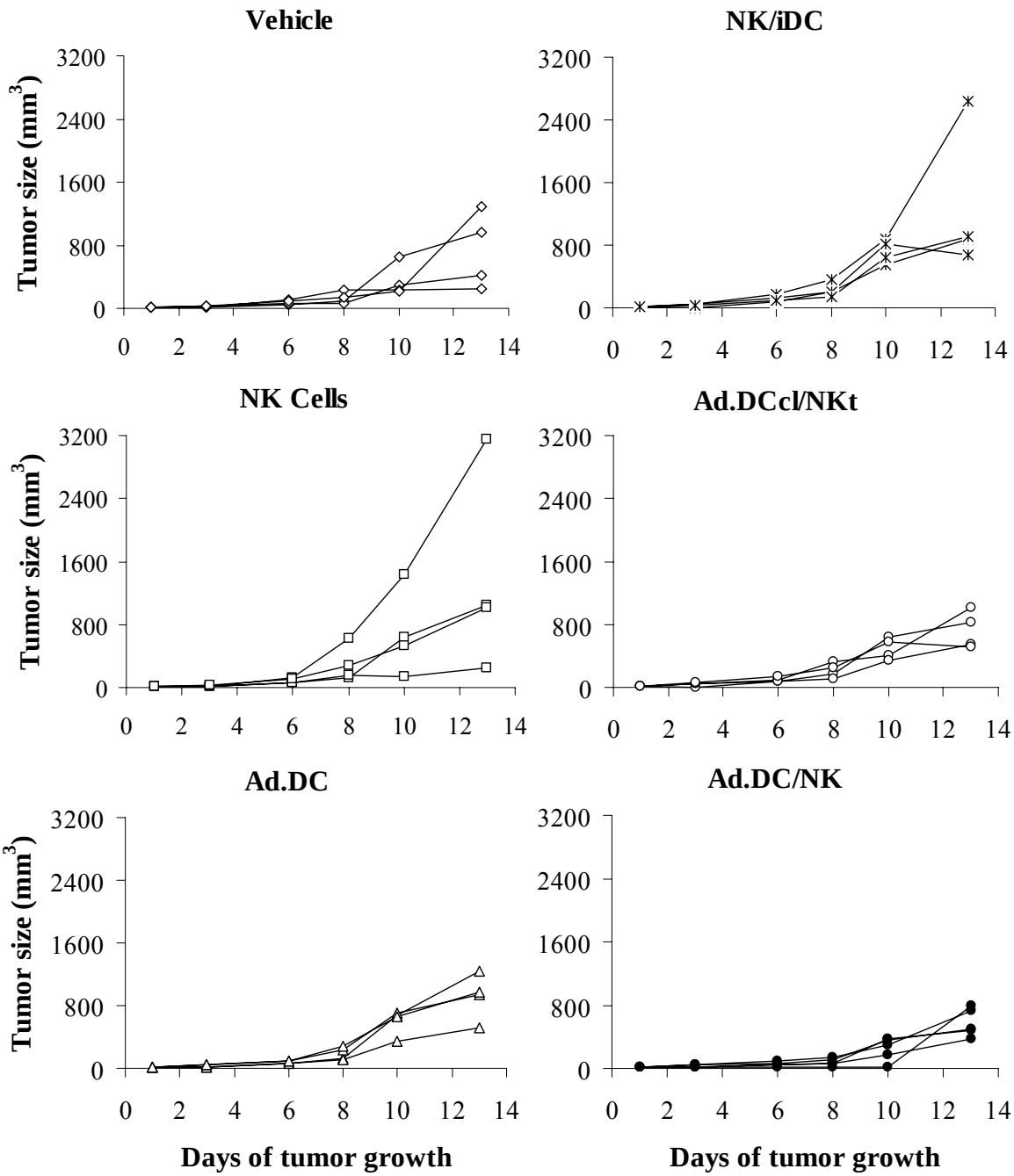

Vehicle
NK/iDC
NK Cells
Ad.DCcl/NKt
Ad.DC
Ad.DC/NK
Tumor size (mm3)
Days of tumor growth
0
800
1600
2400
3200
0
2
4
6
8
10
12
14

Figure S5

% inhibition of tumor growth – Ad.DC/NK vs controls

	Day 6	Day 8	Day 10	Day 13
Vehicle	33	42	31	22
NK	42	74	65	58
Ad.DC	32	60	60	38
iDC/NK	55	66	67	55
Ad.DCcl-NKt	48	64	52	21
Combined controls	**44**	**64**	**58**	**43**

Figure S6

Statistical significance (*p*=) – Ad.DC/NK vs controls

	Day 6	Day 8	Day 10	Day 13
Vehicle	0.247	0.223	0.391	0.511
NK	0.106	0.076	0.118	0.196
Ad.DC	0.222	0.037	0.012	0.066
iDC/NK	0.029	0.019	0.002	0.128
Ad.DCcl-NKt	0.044	0.028	0.038	0.301
Combined controls	**0.016**	**0.030**	**0.023**	**0.201**

Figure S7

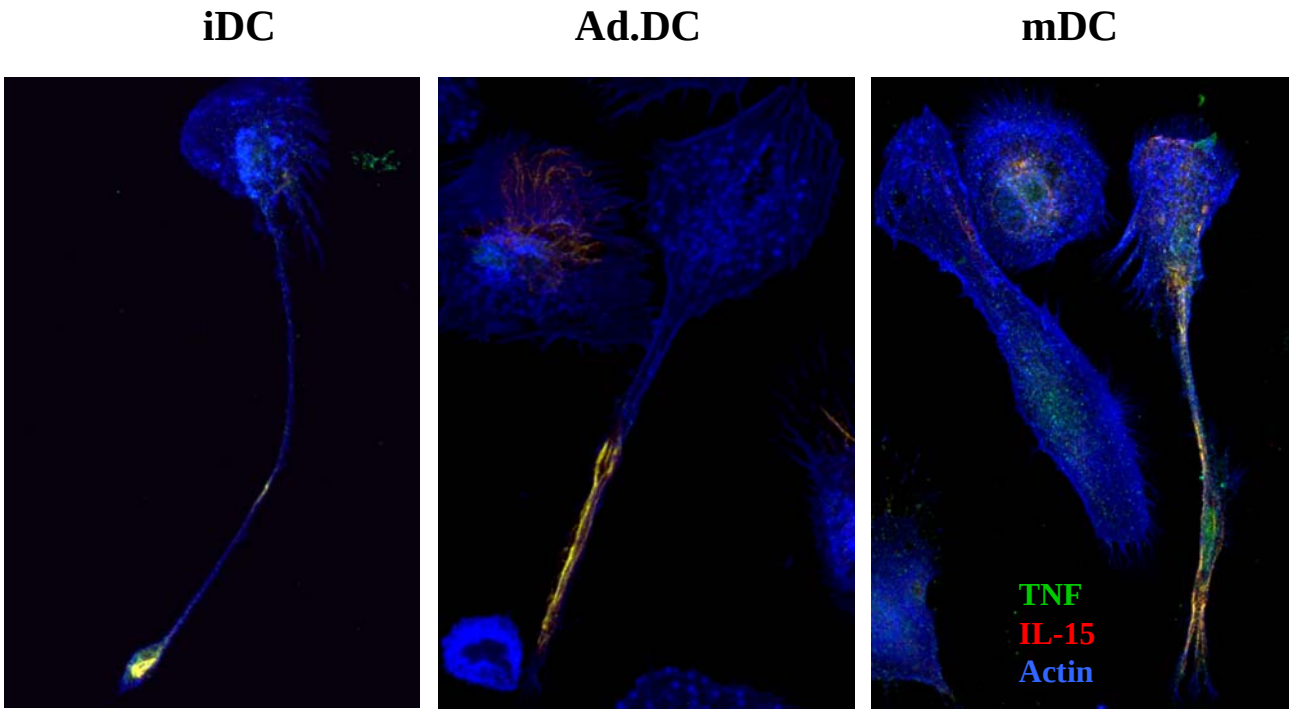


Figure S8

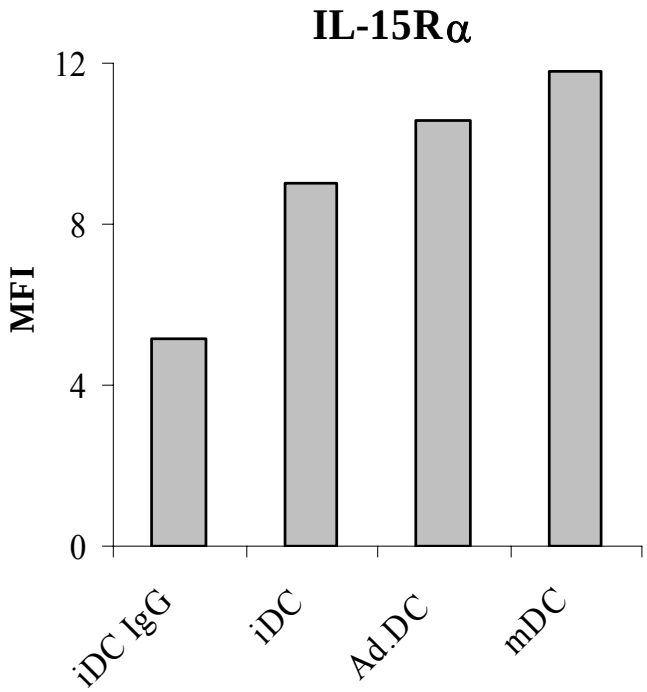


Figure S9

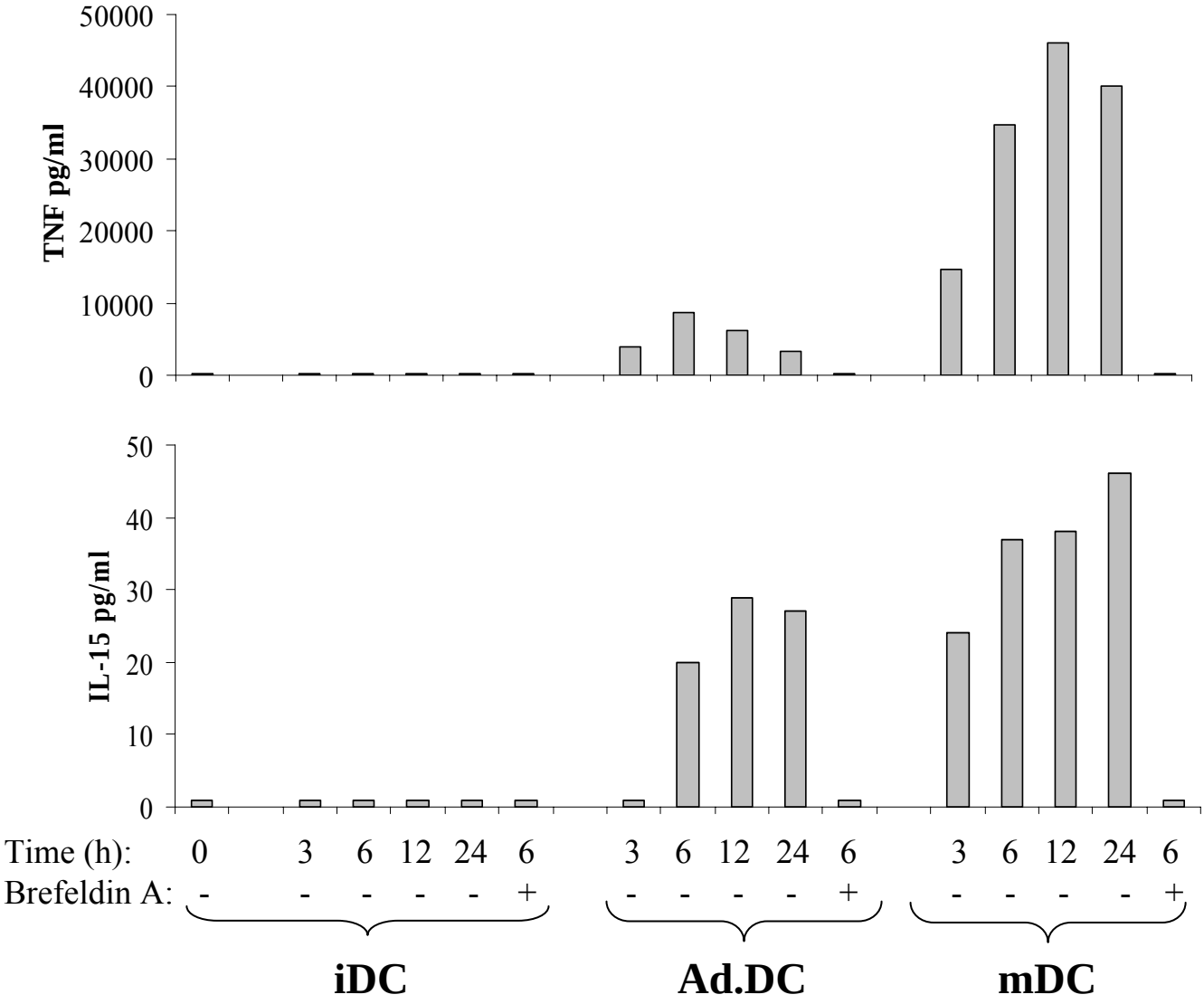

TNF pg/ml
50000
40000
30000
20000
10000
0
IL-15 pg/ml
50
40
30
20
10
0
Time (h): 0 3 6 12 24 6 3 6 12 24 6 3 6 12 24 6
Brefeldin A: - - - - - + - - - - + - - - - +
iDC
Ad.DC
mDC

Figure S10

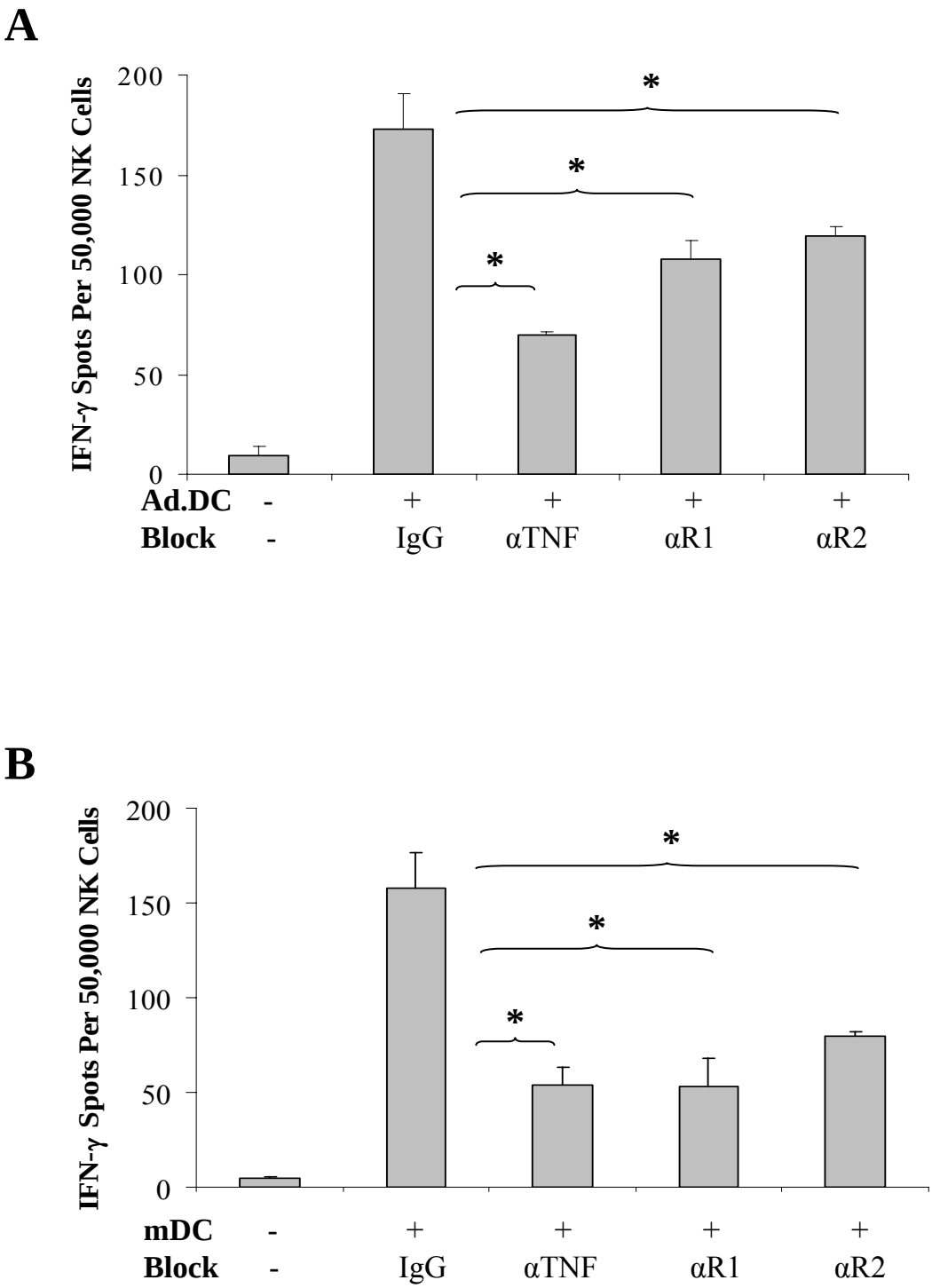
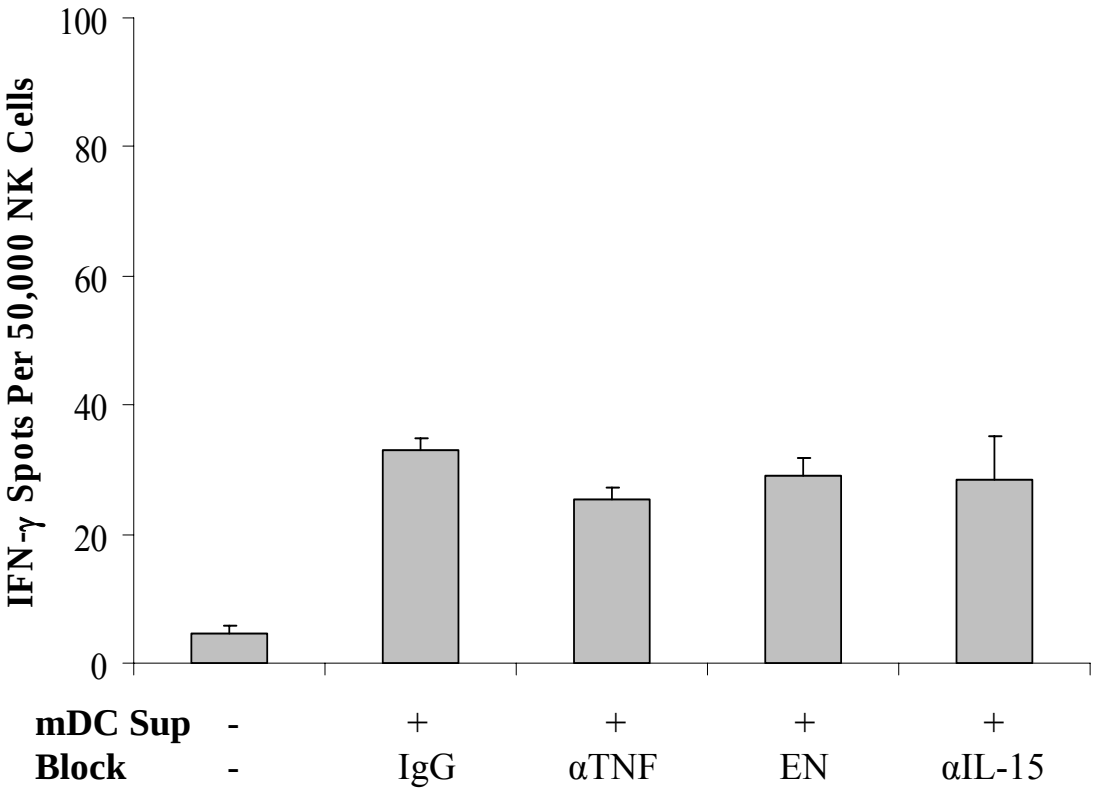

A
IFN-γ Spots Per 50,000 NK Cells
200
150
100
50
0
*
*
*
Ad.DC - + + + +
Block - IgG αTNF αR1 αR2
B
IFN-γ Spots Per 50,000 NK Cells
200
150
100
50
0
*
*
*
mDC - + + + +
Block - IgG αTNF αR1 αR2

Figure S11


IFN-γ Spots Per 50,000 NK Cells
100
80
60
40
20
0
mDC Sup - + + + +
Block - IgG αTNF EN αIL-15