Supporting Information

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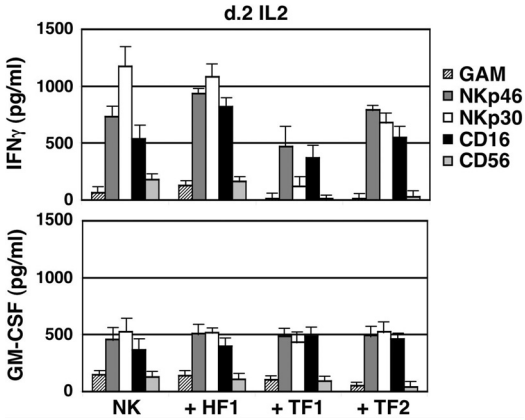

Fig. S1. Evaluation of IFN-γ and GM-CSF production on NK cells that have been cultured with fibroblasts of different origin. NK cells were cultured (in the presence of IL-2) for 2 days either alone or with the indicated fibroblast cell lines and were then stimulated with anti-NKp46, anti-NKp30, or anti-CD16 mAbs. The supernatants were analyzed for their IFN-γ or GM-CSF content. Anti-CD56 mAb was used as control. Bars indicate the value means (± SEM) from three independent experiments.

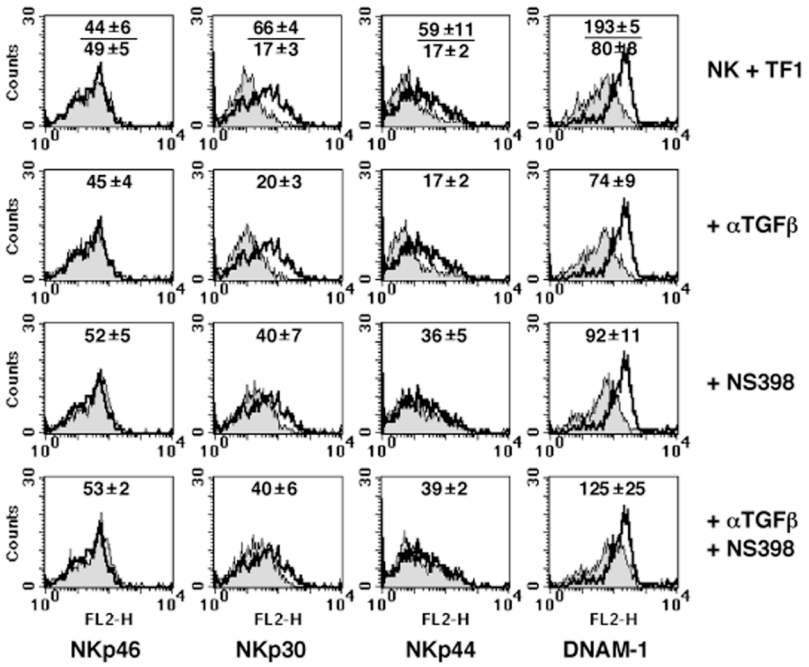

Fig. S2. Effect of anti-TGF-β Abs on the fibroblast-mediated modulation of NK cell phenotype. NK cells were cultured for 6 days in IL-2, either alone or with tumor-derived fibroblasts, and were analyzed by FACS (open and gray profiles, respectively). The inhibitors added in the cultures are indicated. The chicken anti-TGF-β Ab was from R&D Systems.

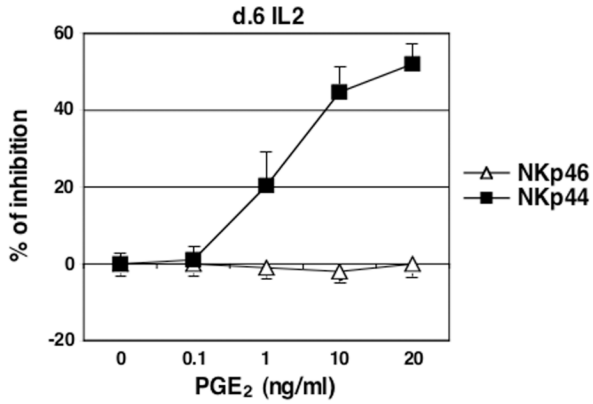

Fig. S3. PGE_2 inhibits IL2-induced NKp44 expression on NK cells. NK cells were cultured for 6 days either in IL-2 alone or in IL-2 plus the indicated doses of PGE_2. NK cells were then analyzed by FACS for the expression of NKp44 or NKp46. Data are presented as percent of NKp44 or NKp46 mean fluorescence intensity (m.f.i.) inhibition induced by PGE_2 and indicate the value means (± SEM) from three independent experiments.

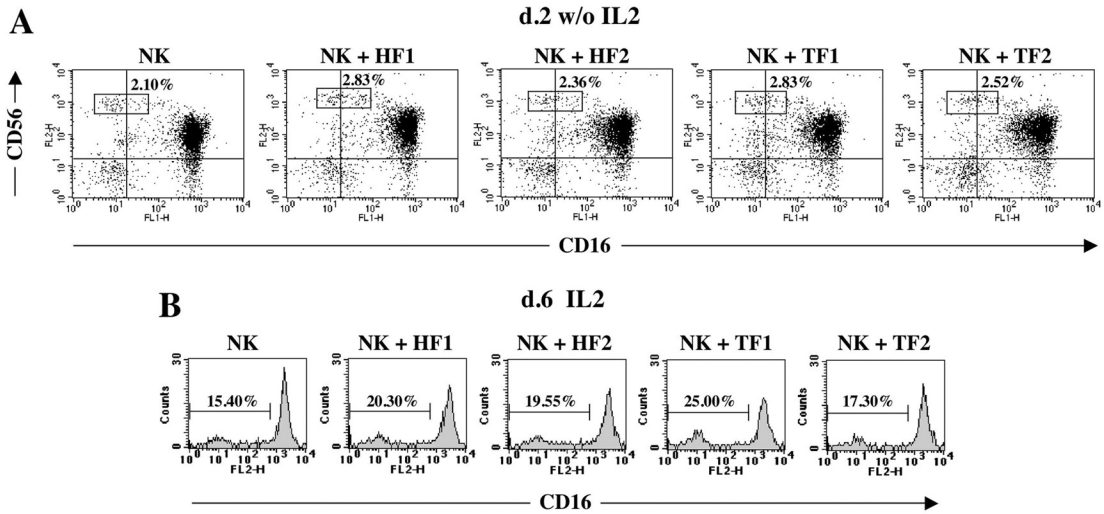

Fig. S4. Evaluation of the CD56^{bright} CD16$^{+/-}$ NK cell subset in NK cell/fibroblast cocultures. (*A*) NK cells were cultured for 2 days either alone or with the indicated fibroblast cell lines, and then analyzed by FACS for the combined expression of CD56 and CD16. (*B*) NK cells were cultured for 6 days in IL-2, either alone or with the indicated fibroblast cell lines, and then analyzed by FACS for the expression of CD16. In *B*, the evaluation of CD56 was not done because the expression of this molecule is up-regulated in NK cells exposed to IL-2.

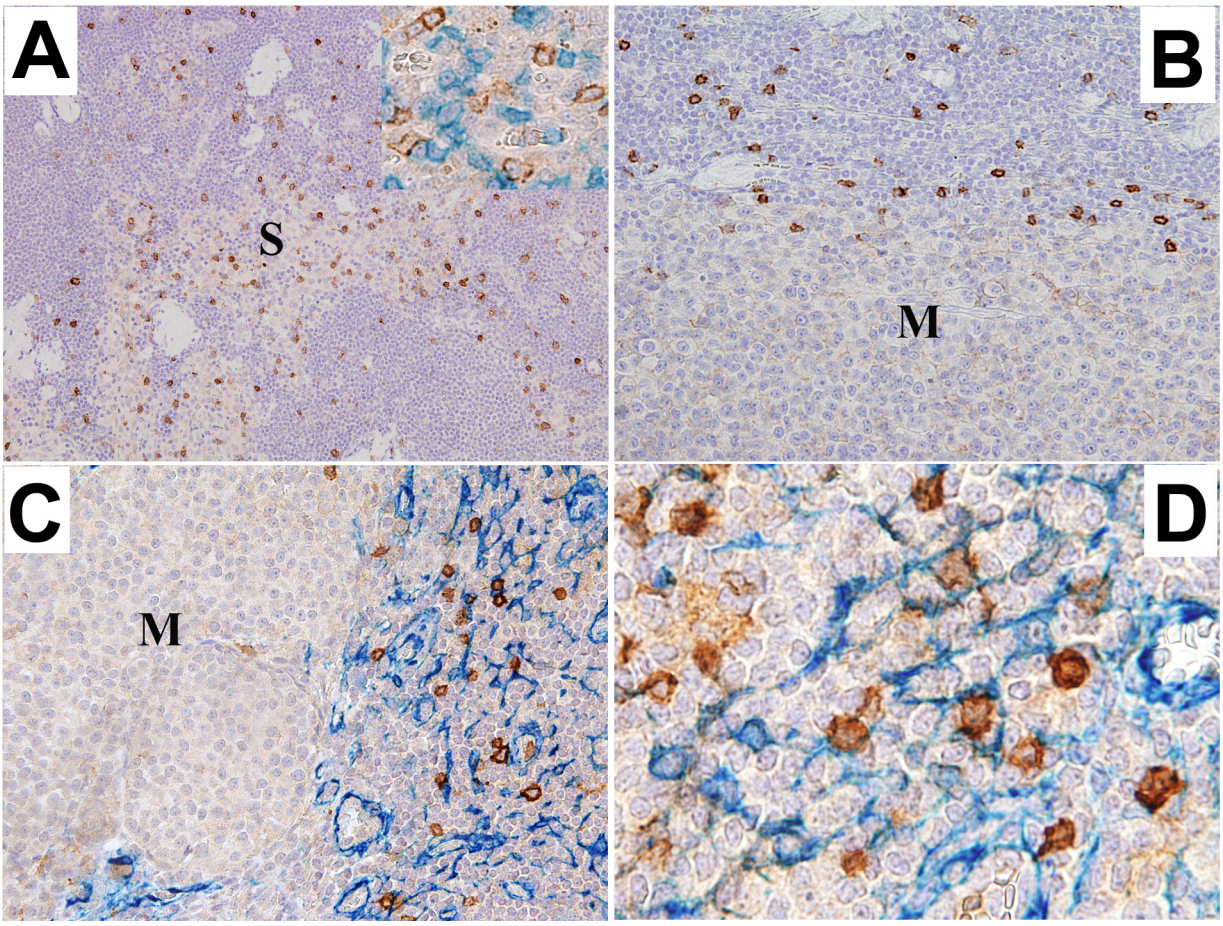

Fig. S5. In nodal melanoma metastases, NK cells are in close vicinity to fibroblasts surrounding the metastatic lesion. Paraffin sections from a metastatic L.N. show numerous CD56$^+$ round-oval cells mainly located in lymphatic sinuses [S] (*A*) and at the periphery of melanoma nests [M] (*B* and *C*). By double immunohistochemistry, CD56$^+$ cells lack CD3 (*Inset* in *A*) and colocalize with fibroblasts (*C*) as shown by the high numbers of cell-to-cell contacts illustrated by high power view (*D*). Sections are immunostained for CD56 (*A*–*D*, brown), CD3 (*Inset* in *A*, blue) and smooth muscle actin (SMA) (*C* and *D*, blue). Original magnifications, ×100 (*A*), ×200 (*B* and *C*), and ×600 (*Inset* in *A* and *D*). Tissue sections (4-μm) were used for immunohistochemical staining using primary antibodies to CD56 (mouse IgG1, clone 123C3.D5, dilution 1:60, 1 h of incubation; Thermo Scientific). Upon microwave antigen retrieval (three times for 5 min at 750 W in EDTA buffer, pH 8.0), reactivity was revealed using NovoLink Polymer Detection System (Novocastra Laboratories Ltd) followed by DAB. Double immunohistochemistry was performed on formalin-fixed tissue sections. After completing the first immune reaction to anti-CD56 (see above), the second immune reaction [to SMA (mouse, clone 1A4; BioGenex) and CD3 (rabbit, clone SP7, dilution 1:100; Thermo Scientific)] was visualized using Mach 4 MR-AP (Biocare Medical), followed by Ferangi Blue (Biocare Medical) as chromogen. The occurrence of NK cells at the site of L.N. melanoma metastasis was analyzed by immunohistochemistry in 15 cases. CD56$^+$ CD3$^-$ cells were regularly found and were particularly abundant in five out of 15 cases.

Table S1. Redirected killing assay on NK cells that have been cultured either alone or with fibroblasts for 6 days without IL-2

	NK without fibro	NK + HF1	NK + HF2	NK + TF1	NK + TF2
No mAbs	2.7 ± 0.3*	4.0 ± 0.6	2.7 ± 0.3	1.3 ± 0.3	0.7 ± 0.3
Anti-NKp46	17.7 ± 1.5	20.7 ± 1.2	18.3 ± 2.4	14.7 ± 2.4	14.7 ± 1.2
Anti-NKp30	20.7 ± 2.8	21.0 ± 1.8	20.3 ± 2.1	16.3 ± 2.6	16.7 ± 3.3
Anti-NKp44	5.3 ± 0.9	6.3 ± 0.9	5.3 ± 0.9	4.7 ± 0.7	4.7 ± 0.3
Anti-NKG2D	5.0 ± 0.6	5.0 ± 0.6	5.3 ± 0.7	4.7 ± 0.3	4.3 ± 0.3
Anti-DNAM-1	4.0 ± 0.6	5.3 ± 0.9	3.3 ± 0.9	3.0 ± 0.6	3.0 ± 0.6

*Data are expressed as % of ^{51}Cr release and indicate the mean ± SEM of three independent experiments.

Table S2. Evaluation of fibroblast-mediated NKp44 modulation and PGE2 production at different NK/fibroblasts ratios

	NKp44 m.f.i. (Ctrl/Fibr)			PGE2 release (pg/ml)		
Fibroblast cell lines	NK/Fibr 1/1	NK/Fibr 2.5/1	NK/Fibr 5/1	NK/Fibr 1/1	NK/Fibr 2.5/1	NK/Fibr 5/1
HF1*	115/107[†]	115/116	115/139	52[‡]	75	65
TF3*	115/20	115/26	115/38	8150	8427	7862
TF6*	115/52	115/51	115/97	946	1710	725
TF10*	115/16	115/32	115/33	9654	9552	9423

*2×10^4 fibroblasts were cocultured for 6 days with NK cells (in the presence of IL-2) at the indicated ratios.
[†]Values indicate the NKp44 m.f.i. referred to: NK cells cultured alone/NK cells cocultured with fibroblasts.
[‡]The supernatants were collected and analyzed by specific ELISA.

Table S3. Cytofluorimetric analysis of IL-2R subunits expression levels in NK cells that have been cultured for 6 days either alone or in the presence of HF1 or TF1 fibroblasts

	NK without fibro	NK + HF1	NK + TF1
Anti-CD25	9*	18	15
Anti-CD122	35	52	50
Anti-CD132	70	72	59

Cell cultures were done in the presence of IL-2.

*Data are expressed as mean fluorescence intensity (m.f.i.) arbitrary units.

Table S4. Viability of NK cells that have been cultured either alone or with fibroblasts for 6 days without IL-2

	NK without fibro	NK + HF1	NK + HF2	NK + TF1	NK + TF2
Viability, %	60*	85	80	79	88

*Data were assessed by trypan blue counts.