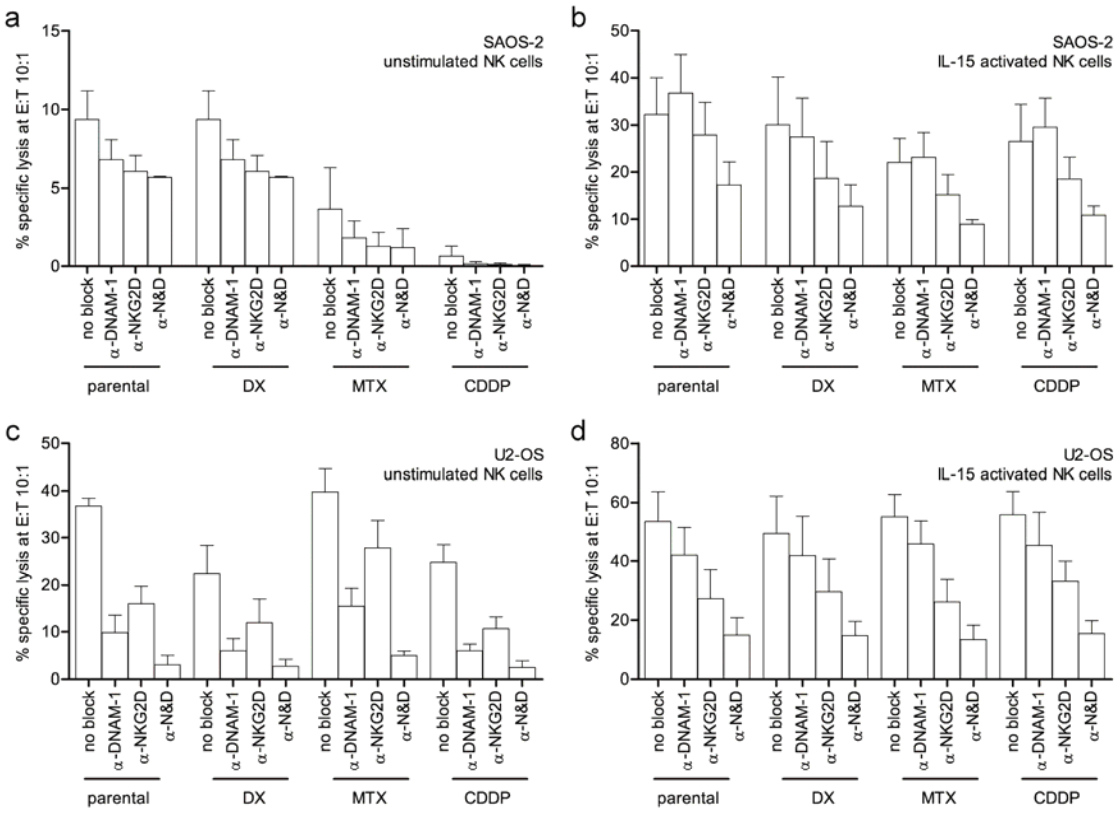
Supplementary figure legends

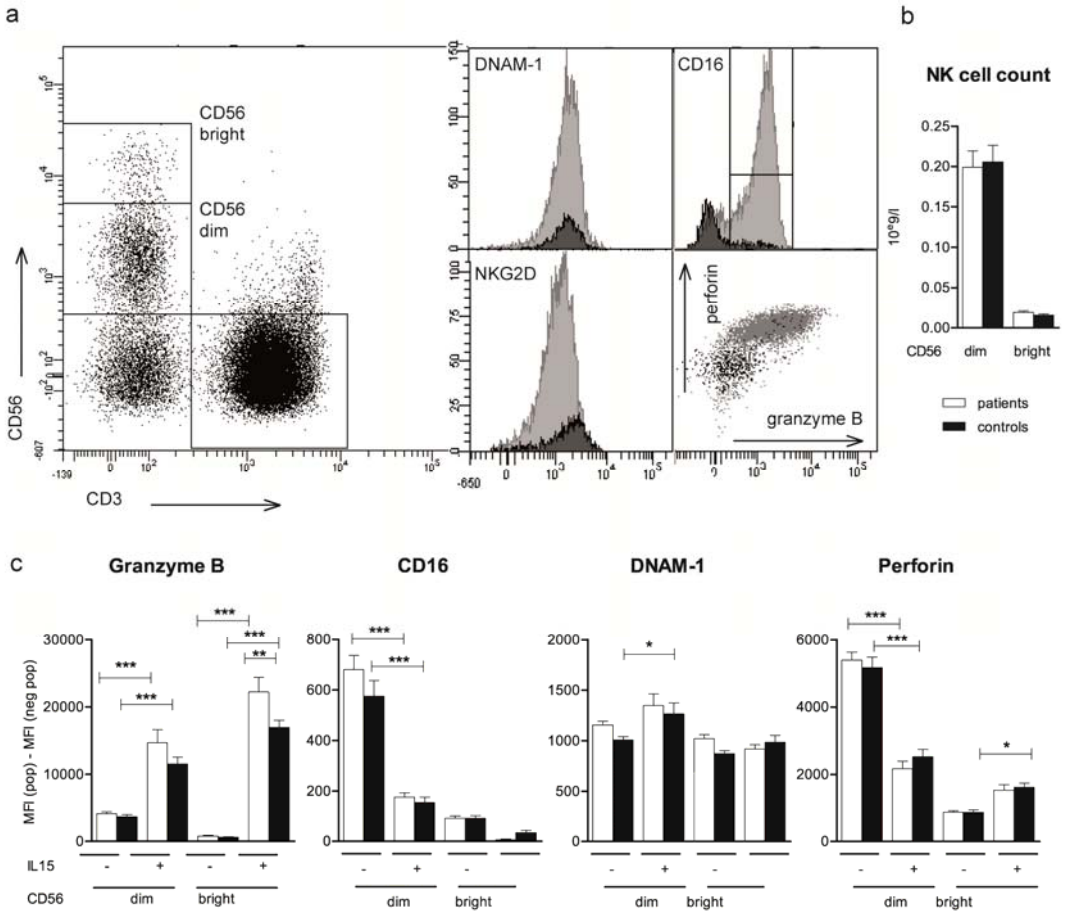
Suppl. Fig.1 The relative contribution of NKG2D and DNAM-1 ligands to the lysis of SAOS-2 and its chemotherapy resistant variants was similar, as demonstrated by blocking DNAM-1, NKG2D or both receptors on unstimulated (**a**) and IL-15 activated (**b**) NK cells. Similar results were obtained in U2-OS (**c** and **d**). Error bars represent standard error of the mean lysis in at least three independent experiments using healthy donor NK cells. DX; doxorubicin resistant variant, MTX; methotrexate resistant variant, CDDP; cisplatinum resistant variant.

Suppl. Fig.2 a, representative example of flow cytometry results of unstimulated peripheral NK cell compartment. The CD3 CD56 plot was gated on lymphocytes based on forward and sideward scatter. Monocytes were gated out by excluding CD14 stained cells (not shown). NK cells were defined as CD3-CD56+ cells and were divided in a CD56 bright and a CD56 dim subset. Expression of NKG2D, DNAM-1, CD16, perforin and granzyme B was determined for CD56 bright (black histogram and dots) and CD56 dim (grey histogram and dots) subsets. **b**, patients and controls had similar numbers of NK cells. **c**, following culture for 3 days in IL15, there was a larger increase in expression level of Granzyme B in NK cells of patients than of healthy controls. Patients and controls had similar levels of CD16, DNAM-1 and perforin on NK cells. In both patients and healthy controls, levels of CD16 decreased in CD56dim and bright NK cells and levels of perforin increased in CD56bright and decreased in CD56dim NK cells upon IL-

15 treatment. ANOVA, *P*-value <0.0001. Bonferroni's multiple comparison post-test; *P*-value <0.05 noted as *; <0.01 = **; <0.001 = ***.



Supplementary figure 1



Supplementary figure 2

Supplementary Table 1: Composition of osteosarcoma tissue array

Number of samples *(of nr of patients)*	*Type of sample*
73 (73)	Pre-treatment samples of the primary tumor
45 (45)	Post-treatment samples of the primary tumor
20 (13)	Lung metastases
3 (3)	Other metastases (2 bone and one lymph node metastasis)
3 (3)	Local relapses
144 (88)	Total samples

Supplementary Table 2: Clinicopathological details of patients in this study

	Tissue array	Peripheral blood NK cell analysis	
		Osteosarcoma patients	*Healthy donors*
Total nr of patients	88	22	23
Age in years: median (range)	15.4 (4-44)	16 (9-56)	20 (8-55)
Sex	n (%)	n (%)	n (%)
Male	47 (53.4%)	10 (45.5%)	11 (47.8%)
Female	41 (46.6%)	12 (54.5%)	12 (52.2%)
Location of primary tumor			
Distal femur	40 (45.5%)	11 (50%)	
Proximal tibia/fibula	27 (30.7%)	4 (18.2%)	
Proximal humerus	10 (11.4%)	4 (18.2%)	
Other long bones	9 (10.2%)	3 (13.6%)	
Axial skeleton	1 (1.1%)	0	
Hand	1 (1.1%)	0	
Histological subtype			
Conventional osteosarcoma	71 (80.7%)	18 (81.8%)	
Osteoblastic	59	12	
Chondroblastic	9	3	
Fibroblastic	3	1	
Unusual	8	2	
Telangiectatic	6 (6.8%)	4 (18.2%)	
High-grade surface osteosarcoma	1 (1.1%)	0	
Small cell osteosarcoma	2 (2.3%)	0	
Date of diagnosis:	Jan 1981- Sep 2003	Feb 2007 - Apr 2008	
Treated according to protocols:	EORTC 80831, 80861 and 80931	Euramos-1	

Supplementary Table 3: Clinicopathological details of primary cultures

Primary culture	*Origin of primary culture*	*Sex*	*Age (y)*	*Histological subtype of osteosarcoma*	*Location of primary tumor*
L2531	Local relapse	Male	17	Osteoblastic	Distal femur
L2808	Lung metastasis	Male	18	Osteoblastic	Distal femur
L2792	Local relapse	Female	31	Fibroblastic	Proximal humerus
L2599	Diagnostic biopsy	Male	13	Osteoblastic	Distal femur
L2635	Diagnostic biopsy	Female	14	Osteoblastic-sclerosing type	Distal femur

Supplementary Table 4: Antibodies used for immunohistochemistry (IHC) and flow cytometry (FC)

Antibody	**Catalog/ clone nr**	**Company**	**Application**
b2-microglobulin	A0072	DAKO (Glostrup, Denmark)	IHC, antigen retrieval (AR): Citrate, 0.01 M, pH 6
HLA-A	HCA-2	Kindly provided by J.Neefjes (NKI, Amsterdam, the Netherlands)	IHC, AR: Citrate, 0.01 M, pH 6
HLA B/C	HC10	Kindly provided by J.Neefjes	IHC, AR: Citrate, 0.01 M, pH 6
MICA	AF1300	R&D systems (Minneapolis, MN)	IHC, AR: Citrate, 0.01 M, pH 6
CD155	HPA012568	Sigma Aldrich (Zwijdrecht, the Netherlands)	IHC, AR: EDTA, 1 mM, pH 8
ULBP-1	HPA007547	Sigma Aldrich	IHC, AR: Citrate, 0.01 M, pH 6
CD112	HPA012759	Sigma Aldrich	IHC, AR: EDTA, 1 mM, pH 8
MICA	MAB1300	R&D systems	FC, cell lines
MICB	FAB1599A	R&D systems	FC, cell lines
ULBP-1	IC1380P	R&D systems	FC, cell lines
ULBP-2	FAB1298A	R&D systems	FC, cell lines
ULBP-3	MAB1517	R&D systems	FC, cell lines
CD48-PE	IM1837U	Beckman Coulter Immunotech (Marseille, France)	FC, cell lines
CD155	IM2755	Beckman Coulter Immunotech	FC, cell lines
CD112-PE	IM3452	Beckman Coulter Immunotech	FC, cell lines
HLA-A/B/C-FITC	555552	BD Pharmingen (San Diego, CA)	FC, cell lines
CD54-PE	555511	BD Pharmingen	FC, cell lines
CD58-PE	555921	BD Pharmingen	FC, cell lines
CD95-PE	340480	BD Pharmingen	FC, cell lines

goat anti-mouse APC	550826	BD Pharmingen	FC, secondary antibody
mIgG1-FITC	639	Beckman Coulter Immunotech	FC, isotype control
mIgG2b	X0944	DAKO	FC, isotype control
mIgG2a	MAB0031	R&D	FC, isotype control
mIgG1-PE	349053	BD Pharmingen	FC, isotype control
mIgG1	MAB002	R&D	FC, isotype control
mIgG2a-PE	349053	BD Pharmingen	FC, isotype control
CD3-PerCPCy5.5	332771	BD Pharmingen	FC, purity of isolated NKs
CD20-FITC	345792	BD Pharmingen	FC, purity of isolated NKs
CD14-APC	340436	BD Pharmingen	FC, purity of isolated NKs
CD56-PE	R7251	DAKO	FC, purity of isolated NKs
CD3-Pacific Blue	558117	BD Pharmingen	FC, PBMCs
CD14-PerCPCy5.5	550787	BD Pharmingen	FC, PBMCs
DNAM-PE	559789	BD Pharmingen	FC, PBMCs
GranzymeB-Alexa700	557971	BD Pharmingen	FC, PBMCs
NKG2D-APC	558071	BD Pharmingen	FC, PBMCs
CD56-PECy7	A21692	Beckman Coulter Immunotech	FC, PBMCs
CD16-FITC	IM0814	Beckman Coulter Immunotech	FC, PBMCs
Perforin-FITC	358-040	Hölzel Diagnostika, Cologne, Germany	FC, PBMCs