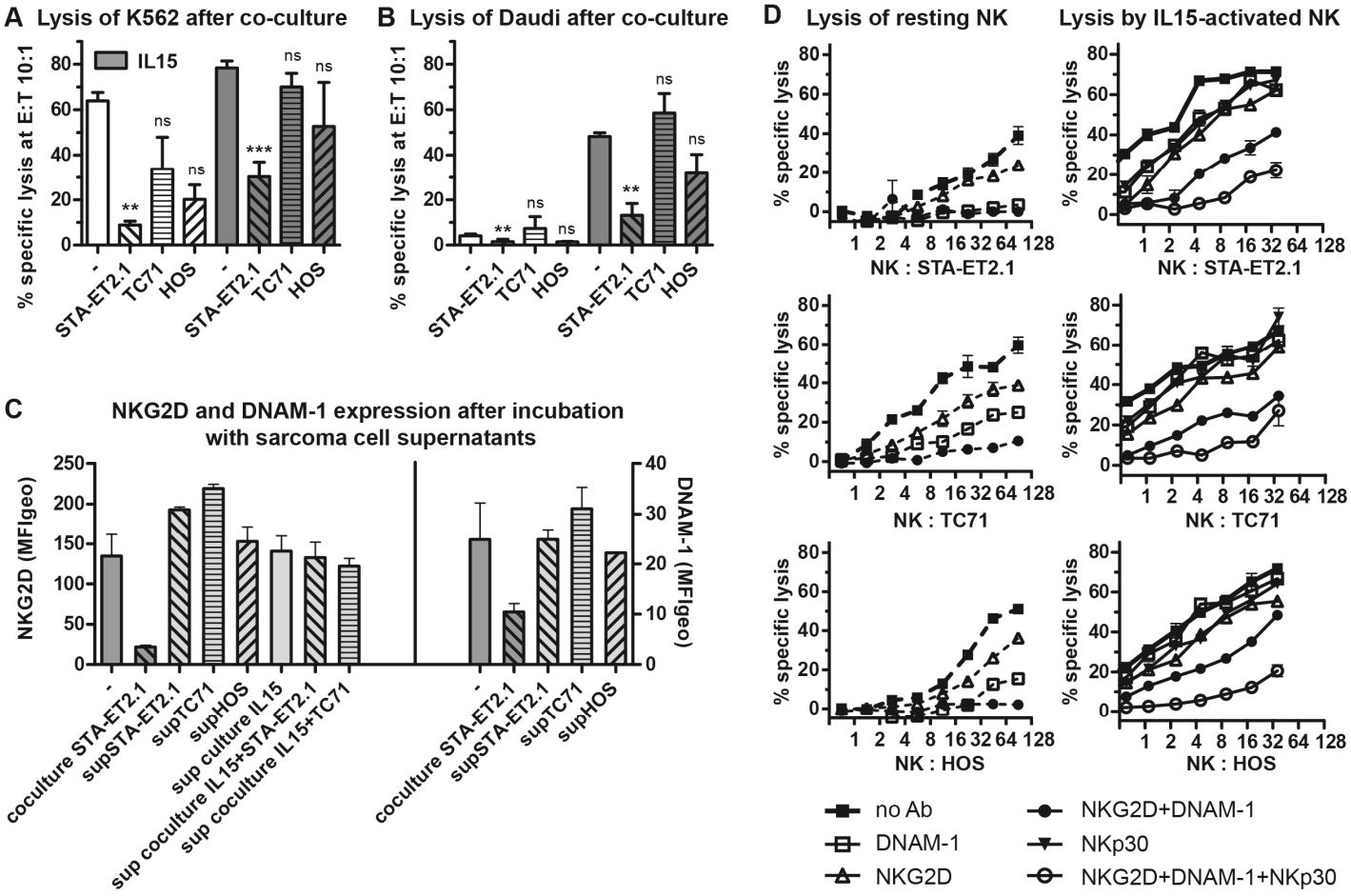
Supplementary table 1

NK cell receptor down-regulation after IL15-stimulated co-culture is not associated with ligand expression on sarcoma cells

	MICA	MICB	ULBP1-3	NKG2D after co-culture	CD112	CD155	DNAM-1 after co-culture	NKp30-Fc	NKp30 after co-culture	NKp44-Fc	NKp44 after co-culture
STA-ET2.1	-	+	+++	17.7%	++	++	42.3%	-	57.3%	-	71.0%
A673	+++	++	+++	38.7%	+++	+++	51.8%	-	n/d	-	n/d
OSA	+	-	++	38.7%	+++	++	47.3%	-	n/d	+	n/d
HOS	++	+++	+++	41.0%	+++	+++	49.3%	+	59.2%	+	74.6%
U2OS	+++	-	+++	47.3%	+++	+++	55.3%	-	n/d	-	n/d
SAOS-2	+++	-	+++	50.3%	++	++	53.3%	-	n/d	-	n/d
CADO-ES	+	+	++	54.0%	+++	+++	87.3%	-	n/d	-	n/d
OHS	-	-	++	55.8%	+	++	42.0%	-	n/d	+	n/d
SK-ES-1	++	+	++	59.8%	+++	+++	64.3%	-	n/d	+	n/d
L1062	+	++	+++	61.7%	+++	+++	77.8%	-	n/d	-	n/d
SK-N-MC	+	+	++	63.7%	+++	+++	68.3%	+	n/d	-	n/d
TC71	-	+	+++	70.1%	++	+++	81.8%	+	86.5%	+	77.2%

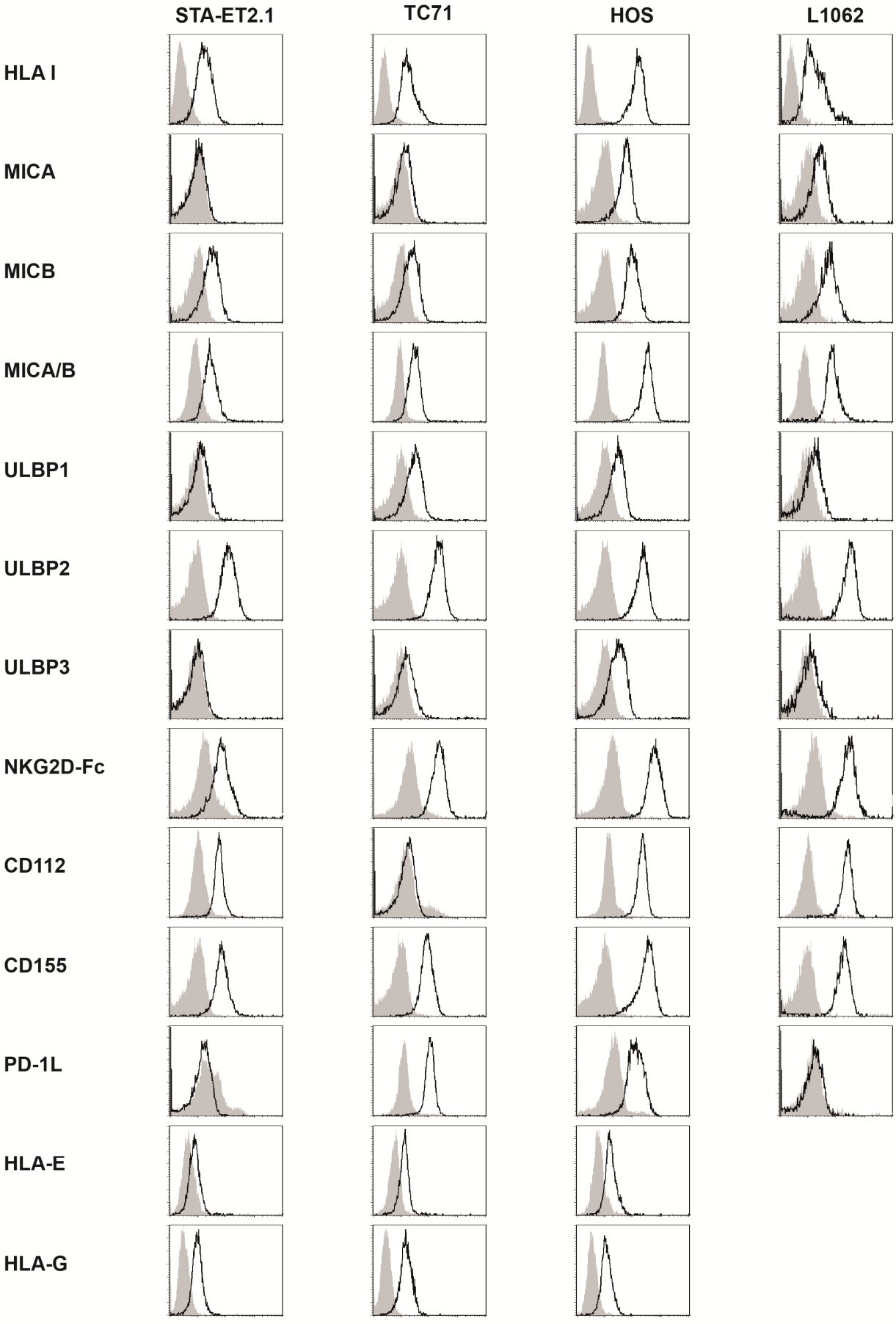
Surface expression of ligands for the NK cell receptors NKG2D (MICA, MICB and ULBP1-3) and DNAM-1 (CD112 and CD155) as derived from supplementary figure 2 and previous data (4, 5, 7). In this study, NKp30 and NKp44 ligand expression, detected by Fc fusion constructs, was investigated. Ligand expression (geometric mean fluorescence intensity (MFI) ratio between ligand staining and isotype control <2 is [-]; MFI ratio >2<5 is [+]; MFI ratio >5<10 is [++]; MFI ratio >10 is [+++]) and expression of respective NK cell receptors (from Fig. 2 and 3; not determined is [n/d]) after IL15-stimulated co-culture (IL15 incubation alone set to 100%) are depicted.

Supplementary figure 1

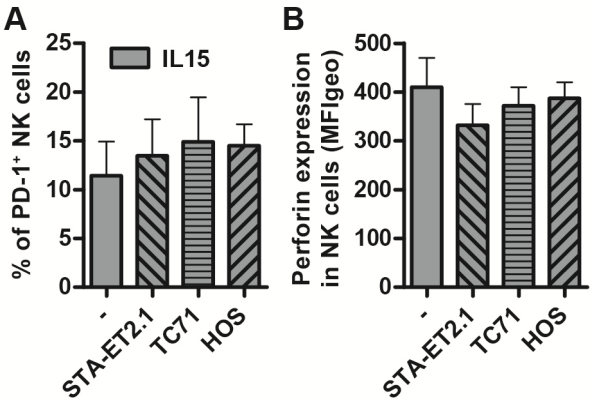


Lysis of K562 (A) and Daudi (B) cells by NK cells after the IL15-stimulated 40 h co-culture of TCD PBMC with sarcoma cells or incubation in medium or IL15 only. (C) Expression (MFIgeo) of NKG2D (left) and DNAM-1 (right) on NK cells after incubation with STA-ET2.1 cells or in medium containing 50% of cell-free supernatants (sup) of long-term, high density cultures of STA-ET2.1, TC71 and HOS cells; or on NK cells after incubation of TCD PBMC in medium containing 33% of supernatant of co-cultures of TCD PBMC and STA-ET2.1 or TC71 cells or IL15 only. (D) Lysis of STA-ET2.1, TC71 and HOS cells by resting NK cells (left) or one week IL15-pre-activated NK cells (right) +/- 10 µg/ml of blocking antibodies against DNAM-1, NKG2D, NKp30 or combinations. Representative data of two or three experiments.

Supplementary figure 2: Expression of NK cell receptor ligands on sarcoma cells



Supplementary figure 3



(A) Percentage of PD-1$^+$ NK cells and (B) intracellular perforin expression in NK cells (MFIgeo) after the IL15-stimulated 40 h co-culture with STA-ET2.1, TC71 or HOS cells. For intracellular stainings, cells fixed with 4% paraformaldehyd and permeabilized with 0.1% saponin. Combined data of two or three experiments.