Supplemental Materials and Methods

Immunohistochemistry

Samples were snap frozen in liquid nitrogen and stored at −80°C. Four-micrometer cryostat sections were fixed in cold absolute acetone for 10 minutes and either immediately used for indirect immunoperoxidase or stored at −20°C up to 6 months with no appreciable changes in reactivity. Slides were incubated overnight with antibodies (50 μg/ml) at 4°C in a moistened chamber. Control sections were incubated with isotype-matched IgG. An indirect avidin-biotin immunoperoxidase staining was performed with commercially available reagents (VECTASTAIN Elite, Mountain View, CA), and the enzymatic activity was developed using 3-amino-9-ethylcarbazole as the chromogenic substrate for 8 minutes. Slices were then rinsed with phosphate-buffered saline, counterstained with Mayer hematoxylin, and mounted with buffered glycerol. At least three nonconsecutive sections from each specimen were analyzed. Normal tissues were from at least two patients and were collected distal to transformed tissues.

Cell Lines

JY and BSM are EBV-immortalized cell lines. Tumor cell lines are listed along with their lineage and HLA-A, -B, and -C typing [1–8]: Molt 4 (T lymphoblastoid; A1, 25; B18, 57; Cw6, 12), Jurkat (T lymphoblastoid; A3; B35), WI-L2 (B lymphoblastoid; A1, 2; B51, 17), Raji (B lymphoblastoid; A3), U937 (myelomonocytic; A3, 19; B51, 18; Cw1, 3), HL-60 (myelomonocytic; A10; B57; Cw6), K562 (erythromyeloid; A11, 31; B18, 40; Cw3), Colo 38 (melanoma; A24, 11; B35, 15; Cw3, 4), SK-MEL-37 (melanoma; A2, 11; B15, 55; Cw1), SK-MEL-93 (melanoma; A2, 31; B21, 35; Cw4), FO-1 and its β_2m transfectant FO-1-β_2m (melanoma; A25; B8; Cw7), A549 (carcinoma; A25, 30; B18, 44; Cw12, 16), BT20 (carcinoma; A24; B15; Cw3, 12), Calu-1 (carcinoma; A2, B40, 41; Cw2, 17), End 9 (carcinoma; A24, 25; B15, 52; Cw1, 3), HT-29 (carcinoma; A1, 24; B35, 44; Cw4), MCF7 (carcinoma; A2, B18, 44; Cw5), T24 (carcinoma; A1; B18; Cw5), HeLa (carcinoma; A68; B15; Cw16), KJ29 (carcinoma; A2; B27; Cw1), and JAR (choriocarcinoma). Early passage cell lines are melanoma Mel20 (A1, 31; B14, 18), ovarian carcinoma Ova7 (A1, 11; B14, 51), endometrial carcinoma End7, and breast carcinoma Br7.

As shown in Table W3 (representative results are shown in Figure W2), all the antibodies with nominal HLA-E specificity detected HLA-E on the surface of 221.AEH transfectants overexpressing a hybrid HLA-E heavy chain capable of self (in *cis*) ligand donation [9], but they failed to detect levels of HLA-E exceeding twice the background in all the other tested cell lines. These include parental 221 cells (expressing HLA-E but lacking HLA-A, -B, and -C and HLA-G), the 221.G1 and 221.B7 transfectants coexpressing "permissive" alleles, and four EBV-immortalized, nontransformed B lymphoid cell lines previously shown to synthesize from intermediate to high levels of HLA-E [10].

Some of the listed tumor cell lines have previously been tested by others [7,11]: U937 and Jurkat (3D12-positive), Raji (3D12-negative), BT-20 and HT-29 (negative for MEM-E/06, MEM-E/07, and MEM-E/08), Calu 1, A549, and K562 (negative for 3D12 and the three MEM antibodies as well), and MCF-7 (negative for the MEM antibodies except MEM-E/07).

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Table W1. Distribution of MEM-E/02–Reactive HLA-E Heavy Chains in Normal Adult Tissues.

Unreactive	Brain, retina, breast, bronchi, epidermis-adnexa, kidney, liver, lung, ovary, peripheral nerves, prostate, skeletal and smooth muscle, stomach, testis, thyroid, urether, urinary bladder
Reactive	Decidual cells, endometrium, epididymis (principal but not basal cells), Kupffer cells, pancreatic ducts, colon-rectum, vascular endothelial cells in parotid, myometrium, pancreas, celomic epithelium, and chorionic villi

Table W2. Expression of MEM-E/02–Reactive HLA-E Heavy Chains in Nonlymphoid Tumors.

Tumor Origin		Positive Histotype(s)
Brain	1/13*	Pilocytic astrocytoma
Breast	4/17	Infiltrating ductal carcinoma
Colon rectum	7/20	Poorly (1)† and medium- (6) differentiated adenocarcinoma (various stages)
Endometrium	7/12	Well- (1) and medium- (6) differentiated adenocarcinoma
Kidney	3/10	Papillary adenocarcinoma
Liver	1/6	Trabecular adenocarcinoma
Lung	11/20	Squamous carcinoma (4), adenocarcinoma (7)
Melanoma (primary)	4/7	Primary lesions (various degrees of dermal invasion)
Melanoma (metastatic)	13/32	
Ovary	9/16	Serous (5) and endometrioid (4) adenocarcinoma
Pancreas	1/5	Well-differentiated adenocarcinoma
Parotid	1/3	Squamous carcinoma
Skin	1/10	Squamous carcinoma
Soft tissues	7/21	Alveolar rhabdomyosarcoma (1), hemangioendothelioma (1), benign (1), and malignant (1), fibrous histiocytoma (1), histocytoid hemangioma (2), cavernous hemangioma (1), osteosarcoma (1)
Stomach	2/11	Poorly (1) and well- (1) differentiated adenocarcinoma
Sympathetic ganglia/adrenal medulla (neuroblastoma)	0/7	
Testis	4/5	Seminoma
Thyroid	2/15	Papillary (1) and follicular (1) adenocarcinoma
Urinary bladder	0/5	
Total	76/230 (33.0%)	

*No. positive/no. tested.
†No. positive cases.

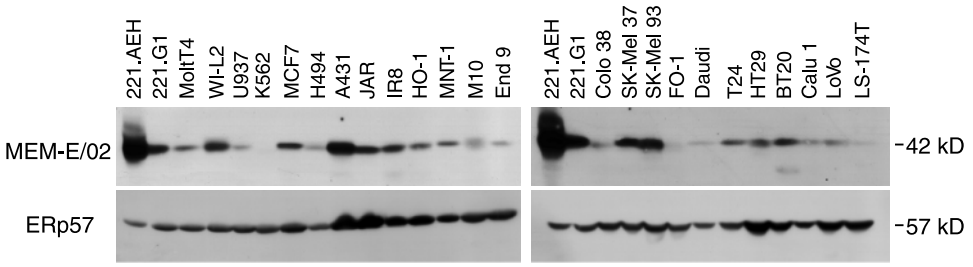

Figure W1. Western blot analysis of neoplastic cell lines. Equal amounts (100 μg per lane) of soluble extracts from the indicated cell lines were run on a SDS-PAGE (10% acrylamide) slab under reducing conditions and electroblotted. Two strips of the filter were stained with MEM-E/02 and a polyclonal antibody to ERp57, respectively. HLA-E accumulation was largely independent from lineage and levels of coexpressed HLA-A, -B, and -C molecules, assessed in a previous study on the same panel [12].

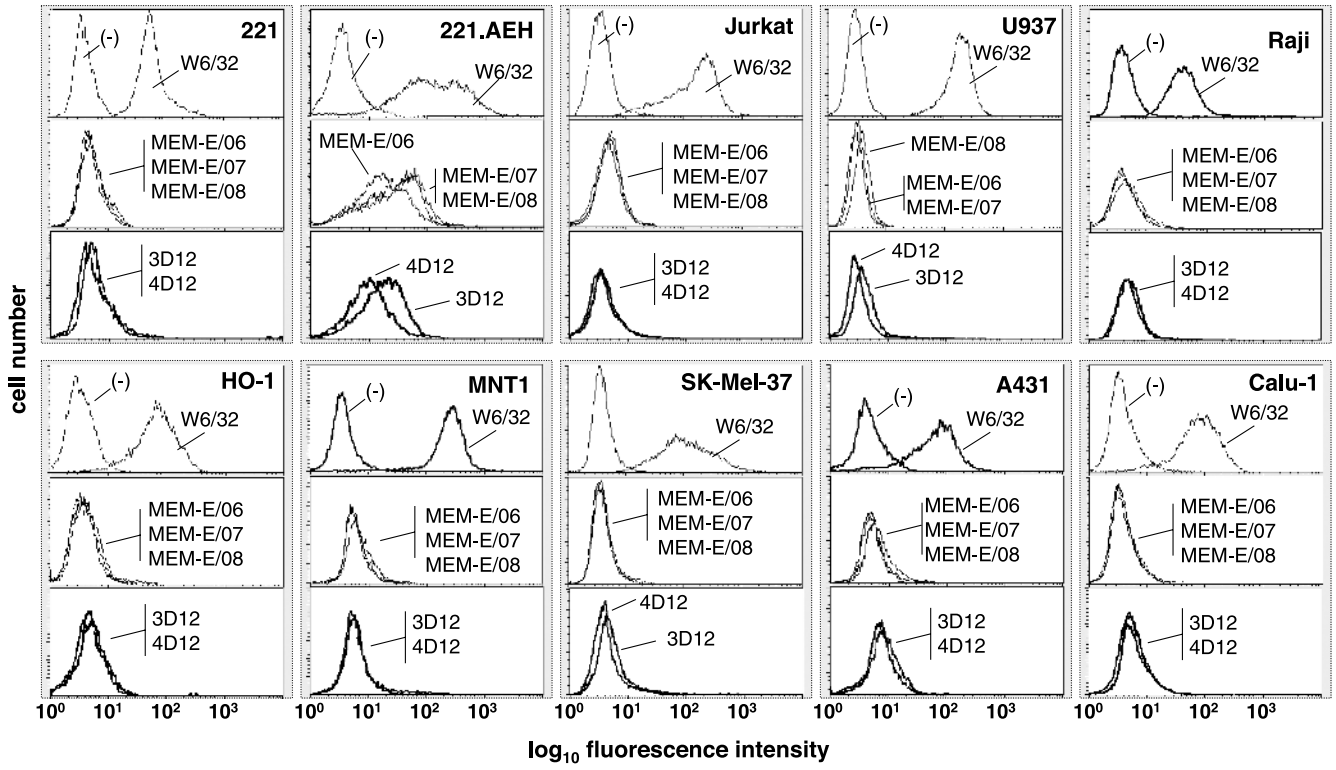

Figure W2. Flow cytometry analysis of the reactivity of MEM-E/02, MEM-E/06, MEM-E/07, MEM-E/08, 3D12 and 4D12. Cell lines (for a complete list and a synopsis see Table W3) were tested for surface reactivity with the MEM antibodies, 3D12, 4D12, and W6/32, as a control. Bound fluorescence was analyzed on a FACScan (Becton, Dickinson & Co). Staining with an isotype-matched (IgG1), irrelevant antibody is shown (−). Each cell line was tested three to five times.

Table W3. Flow Cytometry Analysis of HLA-E Cell Surface Expression with the MEM Antibodies, 3D12 and 4D12*.

Cell Lines		W6/32	MEM-E				3D12	4D12
			/02	/06	/07	/08		
221 and transfectants	221	30[†]	7	3	3	4	2	2
	221.AEH	213	NT[‡]	**20**	**31**	**38**	**25**	**11**
	221.G1	206	4	4	4	3	4	4
	221.B7	73	0	5	1	1	2	2
EBV-B cells	BSM	252	5	1	2	2	NT	NT
	LG2	179	5	2	5	5	NT	NT
	MGAR	364	4	4	4	7	NT	NT
	JY	97	2	2	1	2	2	2
Lymphoblastoid cells	Molt 4	61	3	2	2	1	NT	NT
	Jurkat	124	3	4	3	2	2	2
	WI-L2	149	1	2	0	0	NT	NT
	U937	292	1	3	3	1	4	2
	Raji	88	3	2	2	1	3	2
	K562	17	1	1	1	1	NT	NT
Melanoma cells	Colo 38	75	0	0	1	2	NT	NT
	FO-1	3	2	0	2	1	NT	NT
	FO-1-β_2m	85	0	1	1	1	0	0
	HO-1	41	1	1	1	1	0	0
	IR8	62	0	1	0	1	NT	NT
	M10	42	0	1	0	0	1	1
	MNT-1	141	0	0	0	2	NT	NT
	SK-MEL-37	56	0	0	1	1	5	2
	SK-MEL-93	182	1	1	1	1	NT	NT
Carcinoma cells	A431	81	0	1	2	2	1	1
	A549	111	2	2	1	0	NT	NT
	BT20	14	0	2	0	0	NT	NT
	Calu-1	93	1	2	2	3	0	5
	End 9	9	1	0	0	1	NT	NT
	H494	44	1	2	2	1	NT	NT
	HT-29	32	8	1	2	2	NT	NT
	JAR	6	1	3	3	2	NT	NT
	KJ29-β_2m	179	2	2	5	4	NT	NT
	MCF7	10	0	2	1	4	0	0
	T24	31	1	3	1	2	NT	NT

*Representative results of this analysis are shown in Figure W2.

[†]All the values are expressed as specific mean fluorescence intensity (MFI) after background subtraction. Background values (invariably <9) were calculated by staining in parallel with irrelevant antibodies of the IgG1 and IgG2a isotypes. Specific MFI values represent the mean of three to five separate flow cytometry determinations.

[‡]Not tested.

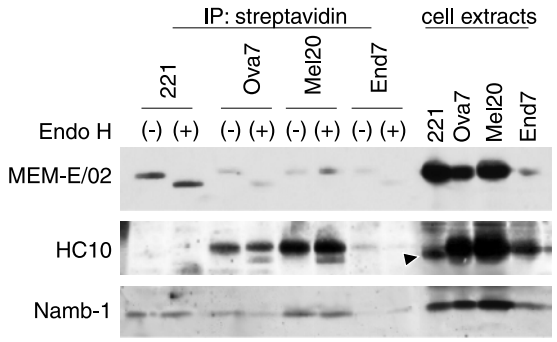

Figure W3. HLA-E expression on surface of early passage tumor cell lines. NP40 extracts from surface biotin-labeling experiments performed as described in Figure 2 were blotted with the indicated antibodies. The arrowhead marks residual staining by MEM-E/02 not removed by stripping.

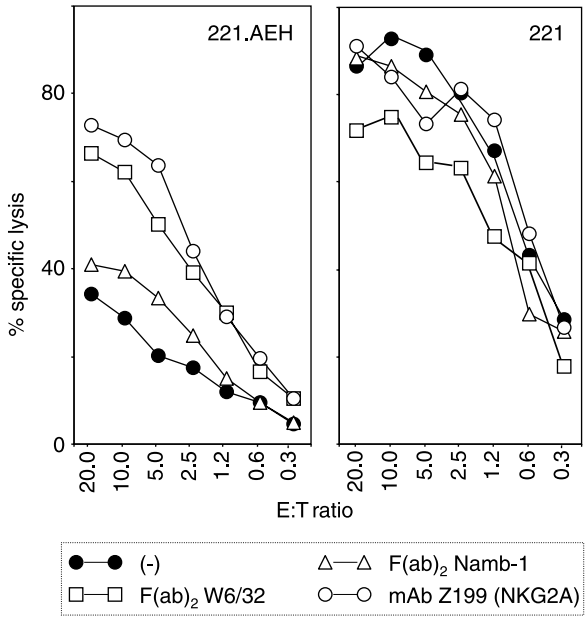

Figure W4. Cytotoxic activity of NKL effectors against 221 target cells. 221 and 221.AEH target cells were tested in parallel, at the indicated E/T ratios, for their susceptibility to NK lysis in a conventional 4-hour ^{51}Cr release assay, using the same seed of NKL cells as effectors, in the absence and presence of the indicated antibodies or $F(ab)_2$ fragments. A representative experiment is shown of six that were performed.

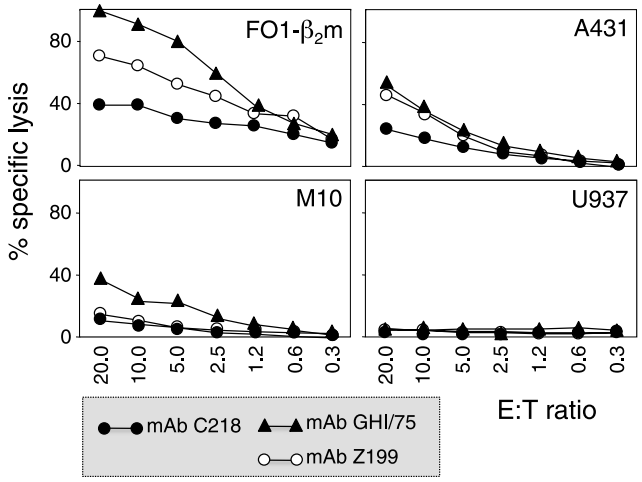

Figure W5. Cytotoxic activity of NKL cells against tumor cells. NKL cells were tested for their ability to lyse the indicated targets in a ^{51}Cr release assay, in the presence of the indicated antibodies (C218 to CD56, GHI/75 to ILT2, and Z199 to NKG2A). Lytic enhancements by GHI/75 and Z199 were statistically significant when the average values of triplicates differed from control more than three times the SD, that is, at E/T ratios from 20:1 to 2.5:1 in FO-1-β_2m and at E/T ratios of 20:1 and 10:1 in A431. All the cell lines were tested three times.

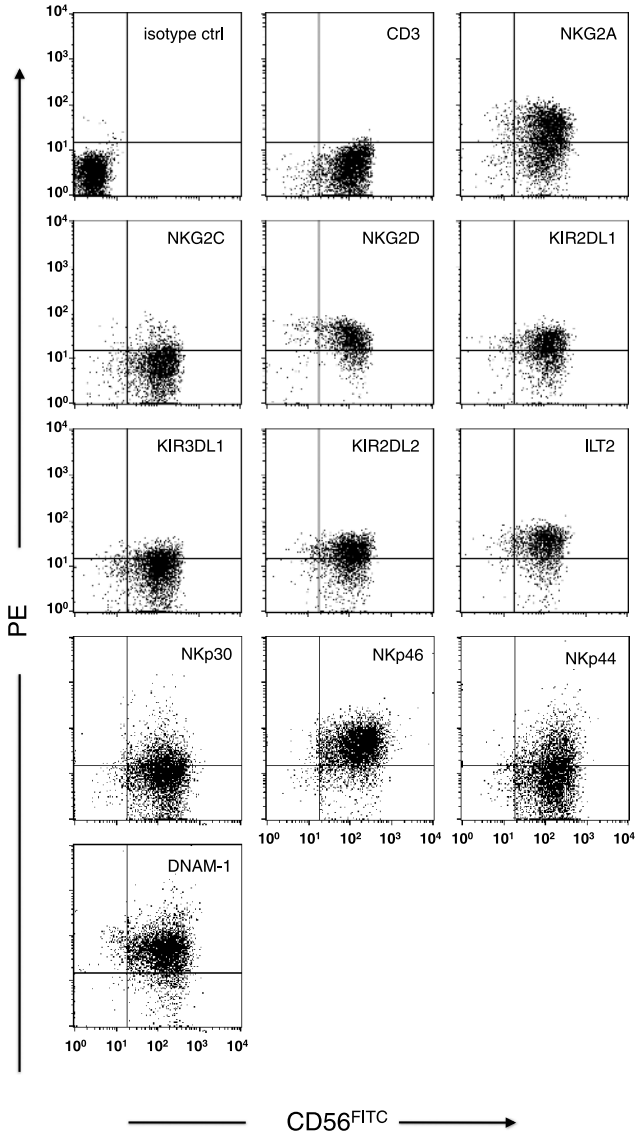

Figure W6. Flow cytometry analysis of polyclonal NK cells. NK cells obtained by negative immunomagnetic selection were submitted to two-color flow cytometry using an fluorescence isothiocyanate–labeled antibody to CD56 (MOC-1), displayed in abscissa, and PE-labeled antibodies to CD3 (UCHT1), NKG2A (131411), NKG2C (134591), NKG2D (149810), KIR2DL1/DS1 (11PB6), KIR3DL1 (DX9), KIR2DL2/DL3 (DX27), ILT2 (GHI/75), NKG2A (131411), NKG2C (134591), NKG2D (149810), DNAM-1 (102511), NKp30 (210845), NKp44 (253415), and NKp46 (195314), displayed in ordinates. A combination of fluorescence isothiocyanate–labeled and PE-labeled isotype-specific controls is shown in the upper right panel. NK cells expanded in culture display a homogeneous CD56 staining intensity that appears to be slightly higher than that of the bulk $CD56^{dim}$ NK cell populations freshly obtained from the PBMCs of two donors before culturing. A slight but consistent enhancement of ILT2, NKG2A, NKG2C, and NKG2D receptors (particularly of NKG2A) was also noted. Receptor expression patterns were similar in all the tested donors with the exception of a variable KIR expression. Among NCRs, NKp30 was consistently less expressed.

Table W4. Expression* of Ligands of Activating NK Receptors by Selected Tumor Cell Lines.

	Antibodies							Ig-fusion			
	MICA	MICB	ULBP1	ULBP2	ULBP3	PVR	Nectin-2	p30-Ig	p44-Ig	p46-Ig	DNAM-1-Ig
Raji	11	1	0	0	0	0	0	0	0	0	1
WI-L2	0	4	0	0	0	0	0	0	1	1	0
A431	10	1	3	8	0	**75**	0	0	0	0	**10**
FO-1-β_2m	**41**	2	0	10	0	**161**	6	**15**	**6**	**6**	**25**
SK-MEL-37	0	3	0	0	2	**145**	0	0	0	0	**19**
Mel20	0	0	0	**19**	0	**52**	0	0	0	0	**7**
Ova7	**14**	0	1	**22**	2	**85**	11	0	**17**	0	**55**
End7	0	0	2	**30**	6	**150**	**14**	0	0	0	**36**
Br7	12	1	0	**27**	5	**43**	**22**	0	0	0	**30**

*Specific MFI following background subtraction.

Surface expression of the ligands of major activating NK receptors NKG2D, DNAM-1, NKp30, NKp44, and NKp46. NKG2D ligands, namely, the MHC class I chain-related antigen A and B (MICA/B), and UL16 binding proteins 1 to 3 (ULBP1-3), were tested by specific antibodies. DNAM-1 ligands were assessed by antibodies to the polyovirus receptor (PVR, CD155) and Nectin-2 (CD112), as well as a chimeric receptor-Ig fusion protein. NKp30, NKp44, and NKp46 ligands were assessed by the respective chimeric Ig-fusion proteins. MFI values greater than 11 and greater than 5 for antibody and Ig-fusion proteins (boldface) are considered positive.