

Supplementary Materials for

The nuclear export protein XPO1 provides a peptide ligand for natural killer cells

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Sci. Adv. **10**, eado6566 (2024)
DOI: 10.1126/sciadv.ado6566

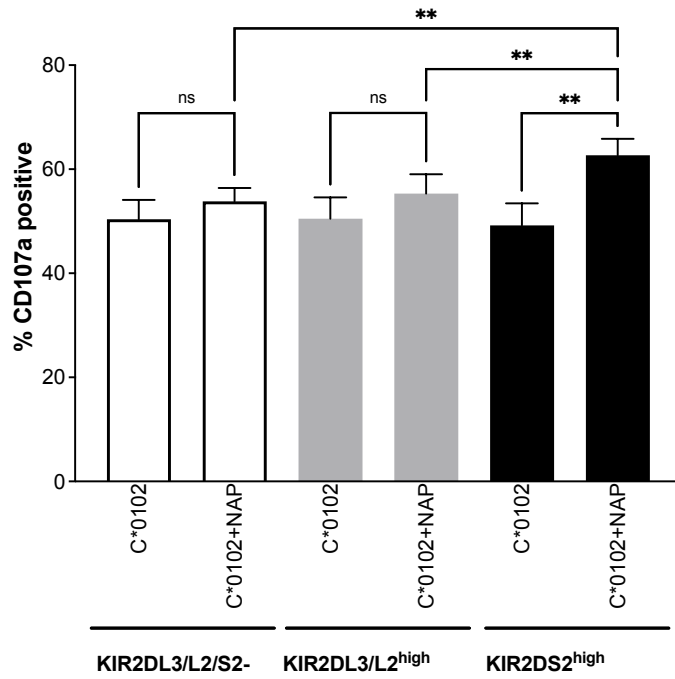
The PDF file includes:

Figs. S1 and S2
Tables S3 to S7
Legends for tables S1, S2, and S8

Other Supplementary Material for this manuscript includes the following:

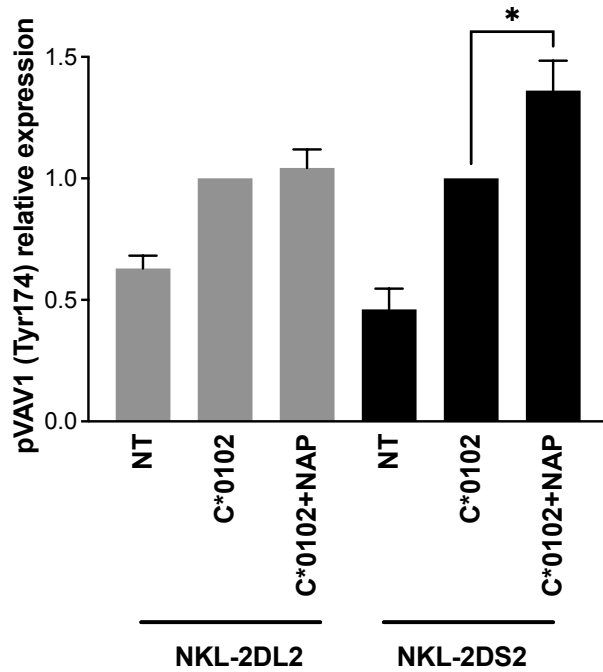
Tables S1, S2, and S8

Supplementary figure S1: KIR2DS2^{high} NK cells are activated by cells expressing HLA-C*0102 in combination with the NAPLVHATL peptide



721.221 cells were transfected with HLA-C*01:02 alone (C*0102) or in combination with the peptide NAPLVHATL (C*0102+NAP), and the cell lines used as targets for degranulation assays with IL-15 activated NK cells as effectors. CD107a expression on the indicated subpopulations of CD3-CD56⁺ NK cells was assessed by flow cytometry against these targets. Summary results from the six donors tested are shown (**p<0.01; ANOVA with Tukey's test for multiple comparisons).

Figure S2: VAV1 phosphorylation of 2DS2-positive NKL cells by NAPLVHATL



NKL-2DS2 or NKL-2DL2 cells were incubated with either no target (NT), 721.221:HLA-C*01:02 (C*0102) or 721.221:HLA-C*0102+NAPLVHATL (C*0102+NAP) cells for 5 minutes and assessed for Vav1 (Tyr174) phosphorylation by immunoblotting, quantified using the ChemiDoc-It Imaging system. Means and standard deviations and standard errors are shown. The NAPLVHATL induces activation of NKL-2DS2 cells but does not change the level of activation of NKL-2DL2 cells (* $p < 0.05$: paired t-test).

Supplementary Tables:

Supplementary Table S1: Peptides identified by mass spectrometry from Huh7 and Huh7:C1 cells (accompanying xls file)

Supplementary Table S2: Quantitation of peptides eluted from Huh7 and Huh7:C1 cells (accompanying xls file)

Supplementary Table S3: Western blot densitometry ratios of pVav1/Vav1 in NKL-2DS2 cells following incubation with the different peptides in Figure 1F

Experiment	No target	No peptide	VAPWNSDAL	LNPSVAATL	NAPLVHATL
1	0.437	0.86158	1.274471	3.04495	5.520958
2	0.239377	1.74814	1.922124	2.844377	2.801711
3	0.325415	1.582443	1.1894527	3.762356	3.12053

Supplementary Table S4: Western blot densitometry ratios of pSyk/Syk in NKL-2DS2 cells following incubation with the different peptides in Figure 1G

Experiment	No target	No peptide	VAPWNSDAL	LNPSVAATL	NAPLVHATL
1	1.808348	1.440003	2.231815	5.684421	5.099912
2	0.366667	0.655251	0.723082	3.968966	3.689665
3	0.8953106	0.8542156	1.8346883	3.426789	4.42731

Supplementary Table S5: CD107a degranulation on CD3-CD56+ NK cells for Figure 3A and Figure S1. Donors were tested against 721.221 cells expressing HLA-C*0102 (C*0102) or HLA-C*0102 plus NAPLVHATL (C*0102+ NAPLVHATL)

Sub-population: Target:	KIR2DL3/L2/S2- negative		KIR2DL3/2hi		KIR2DS2hi	
	C*0102	C*0102+ NAPLVHATL	C*0102	C*0102+ NAPLVHATL	C*0102	C*0102+ NAPLVHATL
Donor 1	31.03	44.23	26.46	47.26	36.03	61.73
Donor 1	31.83	46.23	26.66	46.36	37.03	62.33
Donor 2	40.89	44.39	44.18	36.88	27.46	44.76
Donor 2	39.29	43.69	45.98	35.58	30.06	44.96
Donor 3	55.4	56.2	52.15	57.45	62.73	64.73
Donor 3	54.3	55.6	52.85	57.55	54.63	66.03
Donor 4	57.49	57.69	59.51	60.01	55.04	60.74
Donor 5	58.11	63.21	58.97	69.17	52.63	70.63
Donor 5	56.51	57.91	59.27	61.87	55.23	65.73
Donor 6	65.1	69.6	64.6	72.9	62.65	82.05
Donor 6	64.2	53.4	64.9	63.6	67.85	65.75

Supplementary Table S6: Western blot densitometry data of pVav1 (Tyr174) levels in NKL-2DL2 or NKL-2DS2 cells incubated with 721.221 cells expressing HLA-C*0102 (C*0102) or expressing HLA-C*0102 plus NAPLVHATL as shown in Figure 3C and Figure S2. Data are normalised to C*0102 cells.

Experiment	NKL-2DL2			Experiment	NKL-2DS2		
	No Target	C*0102	C*0102+ NAPLVHATL		No Target	C*0102	C*0102+ NAPLVHATL
1	0.605537	1	0.935796	2	0.47808	1	1.306768
3	0.738612	1	0.948743	4	0.424295	1	1.073959
5	0.678817	1	1.02544	6	0.254012	1	1.814331
7	0.495923	1	1.263223361	8	0.235058	1	1.132993
				9	0.789061	1	1.649083
				10	0.585369	1	1.190711

Supplementary Table S7: Cytotoxicity data for Figure 3F showing the specific cytotoxicity of NKL-2DS2 and NKL-2DL2 against Huh7:C1 cells transfected with siRNA to XPO1 or control siRNA

E:T ratio	NKL-2DS2		NKL-2DL2													
	Control siRNA	XPO1 siRNA	Control siRNA		XPO1 siRNA											
0.5:1	22.9	23.3	14.59	16.96	14.67	13.38	9.78	9.28	13.4	14.8	2.19	4.26	8.57	8.08	4.08	
1:1	36.4	34.5	44.29	24.19	25.86	24.88	31.31	18.58	19.08	12.4	13	36.09	4.16	10.67	8.68	32.01
5:1	58.7	59.2	45.39	53.59	55.26	52.67	51.08	44.38	44.78	42.1	41.7	41.29	10.29	37.97	35.58	37.11

**Supplementary Table S8: Cibersort X analysis of hepatocellular carcinoma samples
from TCGA LIHC dataset (accompanying xls file)**