SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Generation of BND-22

BND-22 was generated by immunizing CD1 mice with human ILT2 (ILT2-Fc and ILT2-HIS; Sino Biological, 16014-H02H, R&D systems, 8989-T2) using hybridoma technology (Precision Antibody). The antibody that demonstrated superior binding and activity was humanized using Germ-Line Humanization (CDR-grafting) technology (Antitope Ltd.). Humanized V region genes were designed based upon human germline sequences with the closest homology to a chimeric version of the antibody and made using gene synthesis. These were then cloned into vectors containing IgG4 (S241P, L235E) heavy chain genes together with human kappa light chain genes. The antibodies and all combinations of CDR-grafted heavy and light chains were expressed in HEK293-EBNA cells. The clone with optimal binding to human and cynomolgus monkey ILT2, blocking and functional activity was chosen for further development as BND-22.

FACS analysis of ILT2 Expression in immune cell subsets

Approximately $5x10^5$ cells per sample were incubated with a commercial ILT2 antibody or PE-conjugated BND-22 in FACS staining buffer (10% human serum in PBS) for 30 minutes. In order to identify different immune cell populations, the following markers were used: CD45, CD4, CD8, CD14, CD11b, HLA-DR, CD33, CD56, CCR7, CD45RA or the appropriate isotype controls (all antibodies from Biolegend). Cell viability was determined with Fixable Viability stain 780 (BD biosciences) and the

cells were analyzed using the Cytoflex flow cytometer (Beckman Coulter). The data were analyzed using CytExpert software (Ver 2.3). The various immune cell populations were defined as detailed in the following table

Immune cells subpopulation	**PBMC**	**Fresh tumors**
Total immune cells	Live CD45+	Live CD45+
CD8 T cells	Live CD45+ CD4-CD8+	Live CD45+ CD4-CD8+
CD4 T cells	Live CD45+CD8-CD4+	Live CD45+CD8-CD4+
T_{EMRA} CD8 T cells	NA	Live CD45+CD8+CD4-CCR7-CD45RA+
NK cells	Live CD45+CD8-CD56+	Live CD45+CD8-CD56+
Monocytes	Live CD45+CD14+ CD11b+	NA
TAM	NA	Live CD45+ CD33+ CD14+HLA-DR+

Bioinformatic analysis

Normalized enrichment score (NES) values for different immune cell types were downloaded from the TCIA database for all TCGA patients. These data where then combined with the individual ILT2 gene expression values for each patient of interest from the TCGA to create a data set that analyze individual cell type enrichment stratified by gene-level expression. In a separate analysis, TCGA RNAseq expression data was analyzed across multiple cancer types. CD8-expressing samples were defined as those with expression above the quartile. The percentage of patients with high T_{EMRA} levels were identified based on high expression (above median) of the T_{EMRA} signature genes KLRG1 and CX3CR1, out of the CD8-expressing samples. In addition, the RNAseq data published by Riaz et al., 2017, Cell, was used to calculate change in expression s of ILT2 in melanoma patients treated with anti-PD-1. An additional analysis was preformed to evaluate ILT2 and PD-1 expression in intra-tumoral CD8 T cells from CRC patients documented in http://crctcell.cancer-pku.cn/.

Binding of BND-22 Human ILT2

Kinetic experiments were performed by Biacore T200 (GE Healthcare) and Evaluation softwareV3.0 (GE Healthcare) in order to evaluate the binding kinetics and affinity between BND-22 to human ILT2 (ILT-His – Novus, 8989T2050; Sino biologics, 16014-H08H; LS-Bio, LS-G97658). A multi-cycle kinetics analysis was preformed using a Protein A capture Sensor chip. A running buffer of HBS-P+ (pH 7.4)

was used and a two-fold dilution range was selected from 25.0 nM to 0.78 nM of ILT2. A 1:1 Langmuir binding analysis was used to analyze the results.

In order to evaluate the binding of BND-22 to membranal ILT2, approximately $5x10^5$ BW-ILT2 cells were incubated with BND-22 conjugated to PE (Biosciences, 703-0005) or with a PE-conjugated isotype control (Biolegend, 400113) in staining buffer (0.05% BSA in PBS) for 30 minutes. Cells were then washed twice using staining buffer and analyzed using the Cytoflex flow cytometer (Beckman Coulter). The data were analyzed using CytExpert software (Ver 2.3).

Binding of BND-22 to other ILT family members

Maxisorp 96 well plates were coated overnight with ILT6-His (Sino Biological,13549-H08H), ILT4-FC (Sino Biological, 4132-H02H) LILRA1-His (Sino Biological, 17220-H08H) in CBC buffer (KPL; cat No C3041) followed by blocking for 1 hour in 1% Casein (Thermo, 37528). BND-22 or positive control ILT4, LILRA1, ILT6 antibodies (Biolegend, 42D1, 338704; R&D systems, MAB3085; Sino Biological, 13549-MM06, respectively) were then added to the plates. A secondary peroxidase-conjugated goat anti human kappa chain (Sigma Aldrich, A7164) or anti-rat antibody (Jakcson, 712-035-153) were added to the plates after washing, followed by detection using TMB. The absorbance was measured using an ELISA Reader (Biotek Synergy-H1 plate reader).

Approximately $5x10^5$ BW-ILT4 cells were incubated with BND-22 conjugated to PE (Innova Biosciences, 703-0005) or with a PE-conjugated isotype control (Biolegend, 400113) in staining buffer (0.05% BSA in PBS) for 30 minutes. Cells were then washed twice using staining buffer and analyzed using the Cytoflex flow cytometer (Beckman Coulter). The data were analyzed using CytExpert software (Ver 2.3). As a positive control for binding to ILT4, an ILT4-binding antibody was included in the assay (Biolegend, 42D1, 338704).

Blocking activity of BND-22

A375-HLA-G were incubated with biotinylated ILT2-FC (Sino Biologics, 1614-H02H; Innova Biosciences, 370-0010) in the presence of BND-22. The binding of ILT2-biotin to the cells was determined using Streptavidin-PE by flow cytometry analysis.

BW cells stably transfected with a chimeric molecule composed of the extracellular portion of human ILT2 fused to mouse z-chain (BW/ILT2-Zeta) were incubated A375-HLA-G or various 721.221 cells in the presence of the BND-22 or a control IgG4 (Biolegend, 403702) or no antibody (medium). The secretion of a mouse IL2 was determined after 48 hours of incubation by ELISA (Biolegend, 431003).

Phagocytosis assays

Monocytes were isolated from buffy coat samples obtained from healthy blood bank donors using a human monocyte enrichment kit (Stem Cells, 15068). The monocytes were grown in RPMI medium supplemented with 10% human serum and M-CSF (50 ng/ml; R&D systems, 216-MC) for 6-7 days in order to generate macrophages. The ability of macrophages to phagocyte tumor cells was examined using a real-time IncuCyte analysis system. Target cell lines were labeled with pHrodo Red Cell Labeling Dye (Satorius, 4649), washed and added to macrophages along with various treatments in replicates (IgG4, Biolegend 403702; BND-22; Erbitux, Merck KGaA; anti-CD47, B6H12). The fluorescence of the pHrodo Red Cell Labeling Dye is increased in an acidic environment such as the one that is resident in the phagosome, thus enabling the quantitation of phagocytosis events by measurement of fluorescence. The IncuCyte instrument (IncuCyte S3 live-cell imaging system; Satorius) sampled the assay plate every 30min for fluorescent red signal intensity and phase images. Phagocytosis events are reflected as accumulation of red fluorescent signal. A threshold of red signal was established for each experiment according to the signal of the tumor cells only wells. The background levels of the tumor cells only were subtracted from the MFI calculated for the different treatments. In order to calculate the present increase from control IgG the following equation was used: 100×(sample-control IgG)/(control IgG)

T cell activity assays

Jurkat wild-type or Jurkat transfected to express human ILT2 (Jurkat-ILT2) (50,000 cells) were co-cultured with mitomycin-treated (30µg/ml) A375 wild-type cells transfected with OKT3scFv-CD14 (A375-WT-OKT3) or A375-HLA-G cells transfected with OKT3 scFv-CD14 (A375-HLA-G-OKT3) (10,000 cells) in the presence of different concentrations of BND-22, an HLA-G-specific antibody (G-0031) or a control hIgG4 (Sino Biologics, HG4K). The amount of secreted human IL2 was evaluated by a commercial ELISA kit following 48 hours.

For MLR assays, dendritic cells generated from monocytes and CD8 T cells (T cells) were isolated from different healthy human donors. The cells were combined in an E:T of 5:1 together with the indicated treatments followed by detection of IFNγ secretion on day 5 by MSD.

For signaling assays, TIL ($120x10^3$ / well) were co-cultured with A375-HLA-G-OKT3 cells in an E:T ratio of 2:1 for 2, 5 and 15 minutes in the presence or absence of BND-22 (20 µg/ml). At each time point, the cells were fixed using 2% PFA (EMS, 15710) followed by permeabilization (Bactlab Diagnostics, 554723). The levels of phosphorylated ZAP70 were evaluated by flow cytometry using antibodies for

CD57, CD28, ILT2 and phospho-ZAP70 (Biolegend 322316, 302906; eBioscience 17-5129-42, 12-9006-41, respectively) or the appropriate isotype controls. Cells were evaluated using the Cytoflex flow cytometer and analyzed using CytExpert software (Ver 2.3).

For ex vivo experiments, single cell tumor suspensions ($0.5X10^5$) were co-cultured with autologous PBMC ($0.5X10^6$) in the presence of IL-2 (50 U/ml; Proleukin Novartis Pharma) and the indicated antibodies (BND-22, human IgG4 Biolegend,403702) and/or anti-PD-1 (Pembrolizumab, Merck MSD) for 5-6 days after which the secretion of TNFa was evaluated by ELISA (Invitrogen, 88-7346-86).

NK cell activity assays

Killing assays were conducted by co-culturing NK cells with different target cell lines including A375 and A253 cells, with or without transfection of HLA-G in the presence of different concentrations of BND-22. Effector NK cells (1.5x105) were pre-incubated for 30 minutes with increasing concentrations of BND-22. Target MHC-I or HLA-G positive target cells at an E:T of 7.5:1 were added and incubated for 5 additional hours at 37°C. IgG4 (Sino Biologics, HG4K) was used as a negative control. Cytotoxicity levels were determined using a fluorometric Lactate dehydrogenase (LDH) detection kit (CytoTox ONE Homogeneous, Promega, G7890) according to the manufacturer's instructions. Fluorescence was measured using an ELISA Reader (Biotek Synergy-H1 plate reader). Percent of specific cytotoxicity was calculated as

$$\% \text{ Cytotoxicity} = \frac{100\ x\ (Test\ sample - Low\ control\ (target\ cells\ only))}{(High\ control\ (target\ cells\ in\ lysis\ buffer) - Low\ control - effectors\ only)}$$

The supernatants were used in parallel for quantification of Granzyme B and IFNγ secretion (Granzyme B – 5 hours; IFNγ – 24 hours) with commercial ELISA kits according to the manufacturers' instructions (Mabtech, 3485-1H-20; Biolegend, 430101, respectively).

Primary NK cells were isolated from buffy coat samples obtained from healthy blood bank donors using a human NK enrichment kit (Stem Cells, 15065). The NK cells were grown in complete RPMI medium supplemented with IL2 (50 IU/ml) for 2 days. On the day of the experiment, the NK cells ($1.5x10^5$/well) were incubated with target cancer cell lines at E:T ratio of 8:1 in the presence BND-22 or control human IgG together with Golgi stop. for 5 hours at 37°C. The levels of intracellular IFNγ and membranal CD107a were evaluated by flow cytometry. Briefly, the cells were stained with the appropriate antibodies for membranal targets including CD3, CD56, CD107a, ILT2 (Biolegend, 317306, 362510, 32862; eBioscience, 17-5129-4, respectively). The cells were than fixed and permeabilized using an appropriate kit (Fixation/Permeabilization kit, Bactlab Diagnostics, 554715) followed by staining of intracellular IFNγ (Biolegend, 506507). Appropriate isotype controls were

used as controls. Viability of the cells was assessed with a viability dye (Bactlab Diagnostics, 565388) followed by analysis with the Cytoflex flow cytometer (Beckman Coulter).

For signaling experiments, NK cells ($120x10^3$ / well) were co-cultured with A253-HLA-G cells in an E:T ratio of 4:1 for 1-15 minutes in the presence or absence of BND-22 (50 µg/ml). At each time point, the cells were fixed by 4% PFA (EMS, 15710) on ice followed by permeabilization (Bactlab Diagnostics, 554723). The levels of phosphorylated ZAP70/Syk was evaluated by flow cytometry using appropriate antibodies including CD56, ILT2 and phspho- ZAP70/Syk (Biolegend 362510; eBioscience, 17-5129-42, 12-9006-41, respectively) , isotype controls and analyzed using the Cytoflex flow cytometer (Beckman Coulter).

FACS analysis of tumor samples from lung lesion metastasis model

Fresh lung samples obtained from the mice were digested into single cell suspensions using an enzymatic mixture of DNase 1 type IV (30U/ml), Hyaluronidase type V (100 µg/ml) and Collagenase (1 mg/ml). Approximately $1x10^6$ cells per sample were incubated with a commercial ILT2 antibody and different antibody mixes (10% human serum in PBS) for 30 minutes. The following markers were used: CD45, CD4, CD8, CD25, CD56, CD69, CD45RA, CD197(CCR7), CD107a, (Biolegend, 368522, 200103, 301006, 302216, 362504, 310912, 304106, 353212, 328626, 329908 respectively) or the appropriate isotype controls. Cell viability was determined with Fixable Viability stain 780. Cells were then washed twice using staining buffer and analyzed using the Cytoflex flow cytometer (Beckman Coulter).

FACS analysis of tumor samples from humanized mice model

At termination, tumors were digested into single cell suspensions and evaluated by FACS analysis for the following markers: 7AAD, hCD45, hCD11b, HLA-DR, ILT2, hCD68, hCD80, hCD206, hCD83, PD-1, hCD45RA, hCCR7, hCD14, hCD56, hCD4 and hCD8.The gating strategy depicted in the following table was used to define the different immune cell populations.

Immune cells subpopulation	**Gating strategy**
Total immune cells	Live CD45+
TAM	Live CD45+CD33+HLA-DR+CD14+
M1 (pro-inflammatory macrophages)	Live CD45+CD33+HLA-DR+CD14+CD80+
M2 (anti-inflammatory macrophages)	Live CD45+CD33+HLA-DR+CD14+CD80-CD83-
DC	Live CD45+CD33+HLA-DR+CD14-
CD8 T cells	Live CD45+ CD3+CD4-CD8+
NK cells	Live CD45+CD3-CD56+

Figure S1

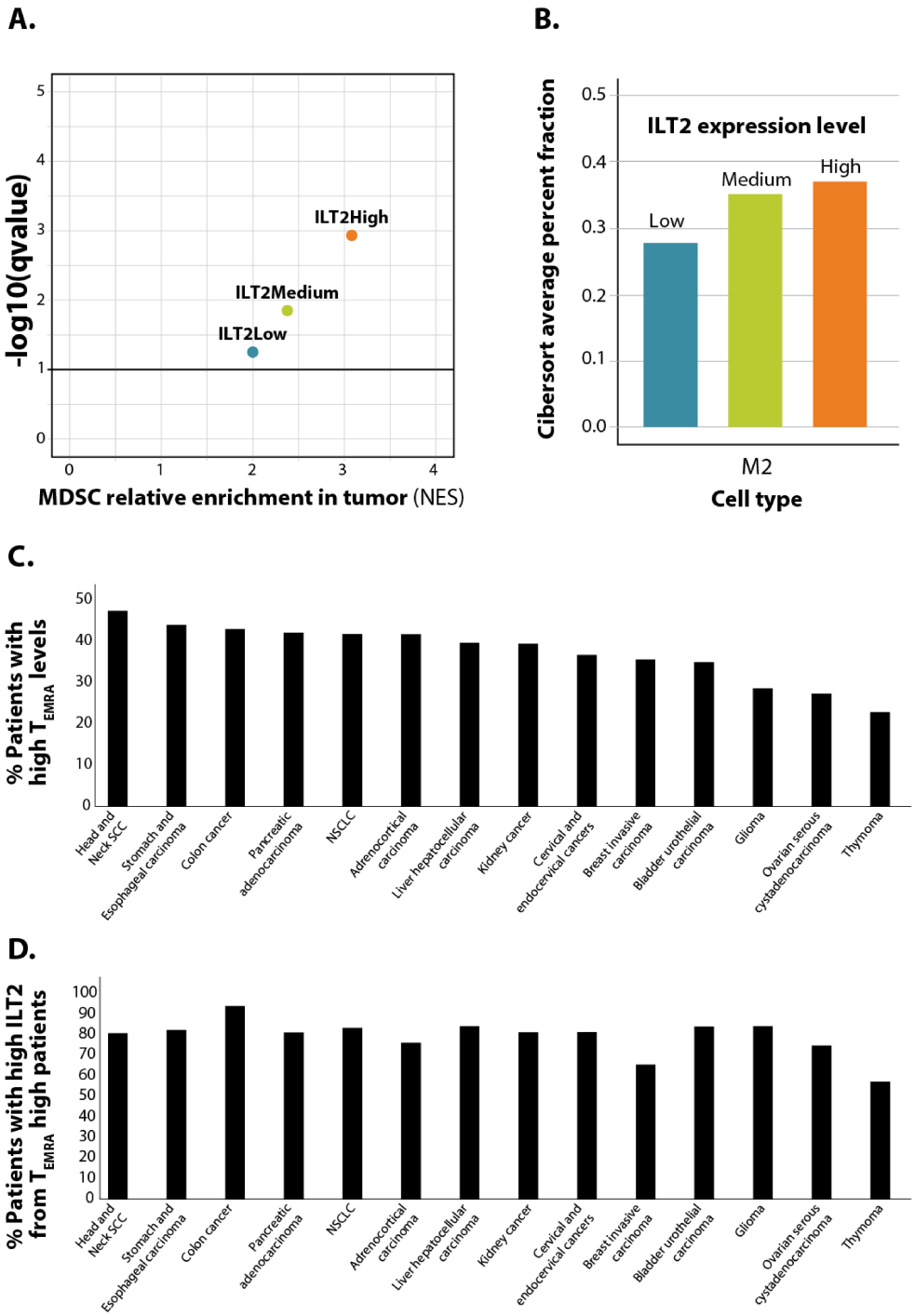
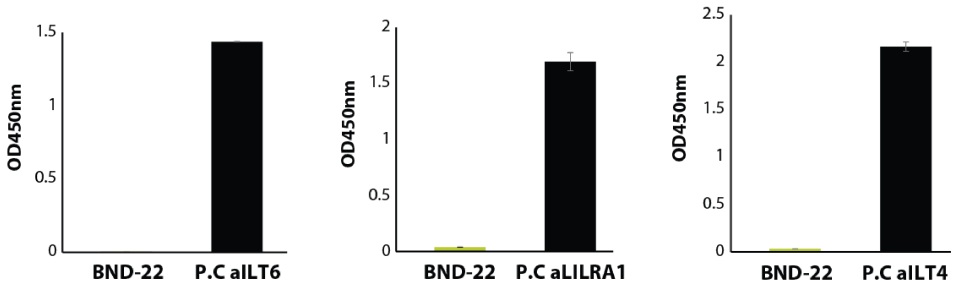


Figure S1: ILT2 expression in the TME is correlated with enrichment of MDSC, M2 and T_{EMRA} CD8 T cells in different cancer indications

(**A**) Normalized enrichment score (NES) values for all MDSC were downloaded from the TCIA database for all TCGA patients. These data where then combined with the individual gene expression values for each patient of interest to create a data set to analyze individual cell type enrichment stratified by gene-level expression. A plot of MDSC enrichment in patients with low, medium and high ILT2 levels is presented. (**B**) Cibersort was used to analyze cell fractionation for M2 cell types analyzed across patients expressing low, medium and high ILT2 levels. An analysis of patients that contained above 25% quartile levels of CD8 was performed for each indication present in the TCGA. (**C**) The levels of TEMRA-specific genes (KLRG1+CX3CR1+) was determined. The graph presents the percent of patients for each indicated cancer type that had high T_{EMRA} levels (above median). (**D**) The percent of patients from the patients presented in **C**. which had high ILT2 levels (above median) is shown.

Figure S2

A.



B.

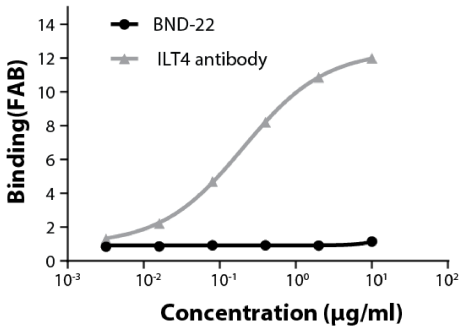


Figure S2: BND-22 shows no binding to ILT6, LILRA1 and ILT4

(**A**) The binding of BND-22 to human ILT6, LILRA1 and ILT4 was determined by an ELISA-based assay in comparison to positive control antibodies which bind these proteins. The absorbance at 450nm with 650nm as a reference is presented. (**B**) The binding to BW-ILT4 cells was evaluated using a PE-labeled BND-22 antibody and analyzed by flow cytometry in comparison to a positive control anti ILT4 antibody. FAB (fold above background) levels of BND-22 or anti ILT4 binding compared to the isotype control at the different concentrations examined were plotted using a 4-parameter analysis. P.C – positive control. P.C ILT6 – anti-ILT6 Ab, P.C LILRA1 – anti-LILRA1 Ab, P.C – anti-ILT4 Ab.

Figure S3

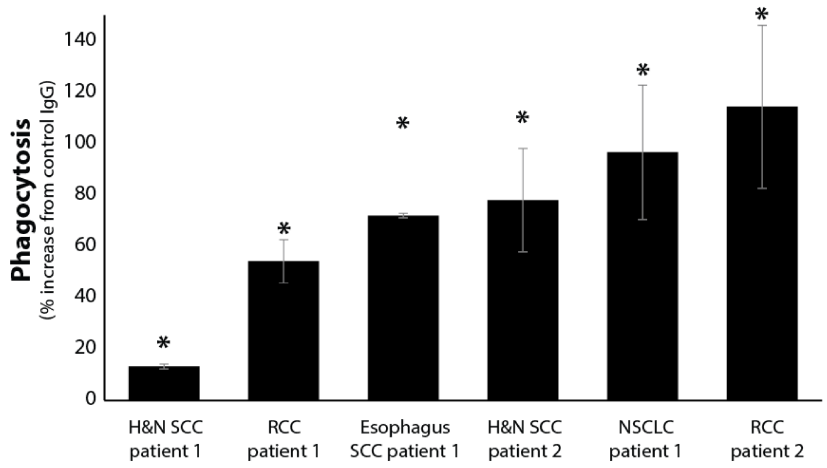
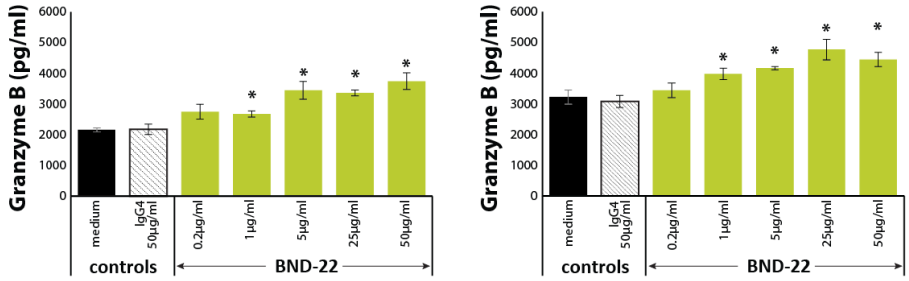


Figure S3: BND-22 enhances the phagocytosis of tumor cells from cancer patients by macrophages

Single cell suspensions were generated by enzymatic digestion of fresh tumors isolated from operations of the indicated patients. The cells were stained with pHrodo Red Cell Labeling Dye, washed and mixed with macrophages generated from monocytes isolated from healthy donors along with BND-22 or control IgG. The percent of phagocytosis increase compared to macrophages and target cells with control IgG at a singular time point is presented. *P<0.05; un-paired Student's T-test compared to cells with control IgG.

Figure S4

A.



B.

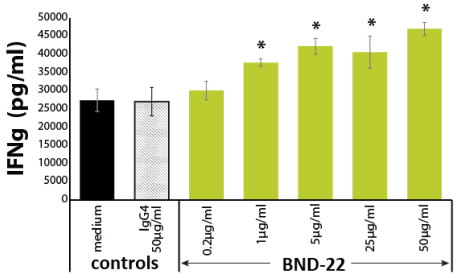
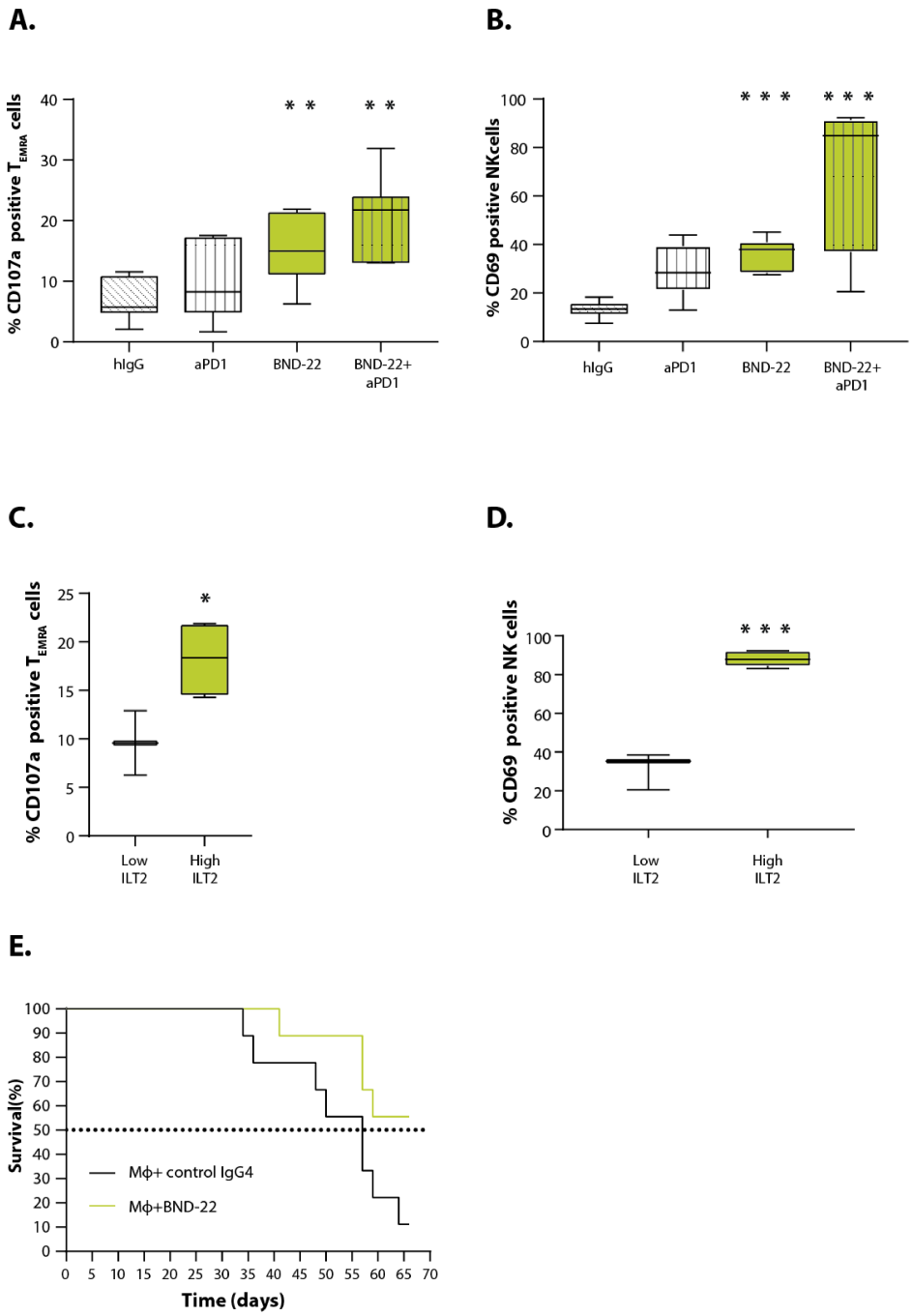


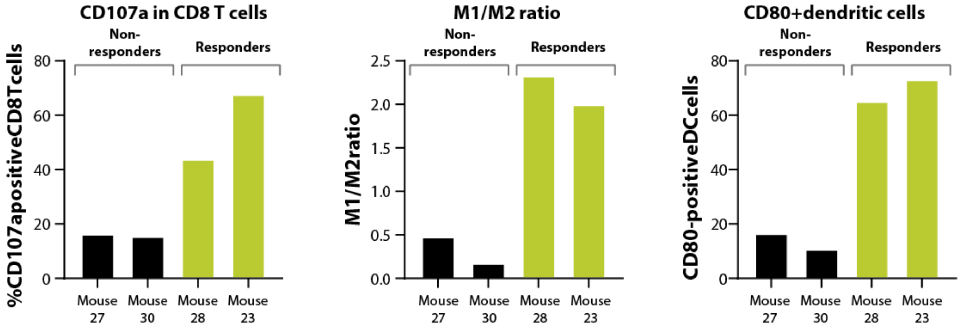
Figure S4: BND-22 enhances Granzyme B and IFNγ secretion of NK cells in the presence of cancer cell lines

NK cells were incubated with the indicated concentrations of BND-22 (batch BND-22-T) or an isotype matched control IgG; Target cells including A375-HLA-G (**A**), A253-WT (**B**) and A253-HLA-G (**C**) were added for additional 5 hours at effector-to-target ratio of 7.5:1. Granzyme B and IFNγ secretion were measured by ELISA. Results represent the mean + S.E of values from 3 repeats per treatment.

Figure S5


A.
% CD107a positive T_{EMRA} cells
40
30
20
10
0
* *
* *
hIgG
aPD1
BND-22
BND-22+ aPD1
B.
% CD69 positive NKcells
100
80
60
40
20
0
* * *
* * *
hIgG
aPD1
BND-22
BND-22+ aPD1
C.
% CD107a positive T_{EMRA} cells
25
20
15
10
5
0
*
Low ILT2
High ILT2
D.
% CD69 positive NK cells
100
80
60
40
20
0
* * *
Low ILT2
High ILT2
E.
Survival(%)
100
90
80
70
60
50
40
30
20
10
0
Mϕ+ control IgG4
Mϕ+BND-22
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
Time (days)

F.



G.

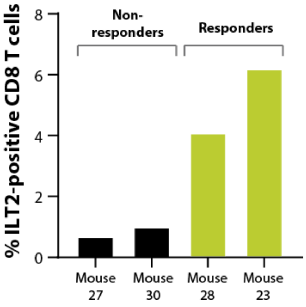


Figure S5: The anti-tumor in vivo activity of BND-22 is mediated by the activation of different immune cells in correlation with ILT2 expression

SCID-NOD mice were engrafted by IV administration with MEL526-HLA-G cells. Human PBMC isolated from healthy donors were administrated to the relevant groups together with IL-2 and the relevant treatments (10 mg/kg each) starting from day 14 (**A-D**). The levels of CD107a in T_{EMRA} T cells (**A**) and CD69 in NK cells (**B**) in the lungs of the treated mice. CD107a (**C**) and CD69 (**D**) expression in BND-22 treated T_{EMRA} or NK cells in mice that received PBMC from donors with low or high levels of ILT2 in their T_{EMRA} cells or NK cells, respectively. The results represent the average of tumor weight ±SE from 5-9 mice per treatment group. Un-paired T test was used to calculate statistical significance between the groups * $P < 0.05$. ** $P < 0.005$. *** $P < 0.0005$. SCID-NOD mice were engrafted SC with COLO-320-HLA-G cells in parallel to injection of macrophages generated from 3 healthy donors. The animals were treated with BND-22 or with a control human IgG4 SC on day 0 followed by 4 additional injections IV. (**E**). Survival analysis was preformed using a Kaplan-Meier curve. Log-rank (Mantel-Cox) test was used to was used to calculate statistical significance in survival between the groups * $P < 0.05$. Humanized NSG mice were engrafted with CD34+ immune cells from human donors. After 14 weeks, the mice received subcutaneous injections of A253-HLA-G cancer cells. Once the tumors were established (80mm^3), the mice were administered IV with BND-22 or control hIgG4 twice a week. (**F**). At day 46 the mice were sacrificed, and the levels of different markers were evaluated by FACS analysis. CD107a in CD8 T cells, M1/M2 ratios and the levels of CD80+ dendritic cells in the tumors of the indicated mice are presented. (**G**) Baseline ILT2 levels in peripheral CD8 T cells of the indicated mice.

Table S1: Rate constants for the modified peptides of ILT2 derived from HRF and trypsin digestion

Position in ILT2	Sequence of ILTS SEQ ID NO: ( )	Free ILT2, *K*free, s-1	ILT2-BND-22com, *K*com, s-1	Ratio, *K*free/*K*com	NR Ratio/1.44
5-24	PTLWAEPGSVITQGSPVTLR (22)	8.93 ± 1.86	7.04 ± 1.24	1.27	0.88
25-35	CQGGQETQEYR (23)	1.18 ± 0.11	0.77 ± 0.070	1.53	1.06
42-48	TALWITR (24)	2.62 ± 0.50	1.56 ± 0.26	1.68	1.17
49-55	IPQELVK (25)	2.41 ± 0.42	1.5 ± 0.24	1.61	1.12
56-71	KGQFPIPSITWEHAGR (3)	9.09 ± 0.87	2.31 ± 0.17	3.94	**2.74**
57-71	GQFPIPSITWEHAGR (4)	6.93 ± 0.68	1.97 ± 0.38	3.52	**2.44**
74-83	CYYGSDTAGR (26)	0.58 ± 0.057	0.32 ± 0.078	1.81	1.26
84-100	SESSDPLELVVTGAYIK (5)	1.26 ± 0.15	0.37 ± 0.055	3.41	**2.35**
101-134	PTLSAQPSPVVNSGGNVILQCDSQVAF DGFSLCK (27)	5.46 ± 0.85	11.08 ± 1.94	0.49	0.34
135-151	EGEDEHPQCLNSQPHAR (28)	5.62 ± 0.81	6.81 ± 1.08	0.83	0.58
156-167	AIFSVGPVSPSR (29)	1.51 ± 0.20	1.0 ± 0.13	1.51	1.05
156-168	AIFSVGPVSPSRR (30)	1.6 ± 0.17	0.94 ± 0.013	1.7	1.18
168-172	RWWYR (31)	0	0	-	-
173-200	CYAYDSNSPYEWSLPSDLLELLVLGVSK (32)	7.67 ± 0.73	5.39 ± 0.57	1.42	0.99
201-230	KPSLSVQPGPIVAPEETLTLQCGSDAGY NR (33)	4.24 ± 0.37	2.64 ± 0.19	1.61	1.12
236-265	DGERDFLQLAGAQPQAGLSQAN*FTLG PVSR (34)	4.55 ± 0.84	3.21 ± 0.40	1.41	0.98
240-265	DFLQLAGAQPQAGLSQAN*FTLGPVSR (35)	3.55 ± 0.70	2.37 ± 0.11	1.5	1.04
240-265	DFLQLAGAQPQAGLSQ ANFTLGPVSR (36)	1.96 ± 0.24	1.55 ± 0.17	1.26	0.88

266-272	SYGGQYR (37)	0	0		-
273-301	CYGAHNLSSEWSAPSDPLDILIAGQFYD R (38)	5.83 ± 0.83	5.16 ± 0.82	1.13	0.78
336-344	EGAADDPWR (39)	5.37 ± 0.69	5.33 ± 0.98	1	0.69
354-372	YQAEFPMGPVTSAHAGTYR (40)	23.01 ± 6.49	15.19 ± 1.98	1.51	1.05
373-380	CYGSQSSK (41)	0	0	-	-

Table S2: Rate constants for the modified peptides of the ILT2 derived from HRF and trypsin/Asp-N digestion

Position	**Sequence SEQ ID NO: ( )**	**Free ILT2, *K*free, s-1**	**ILT2-BND-22com, *K*com, s-1**	**Ratio, *K*free/*K*com**	**NR Ratio/1.53**
5-24	PTLWAEPGSVITQGSPVTLR (42)	15.07 ± 1.16	19.66 ± 1.7	0.78	0.51
10-24	EPGSVITQGSPVTLR (43)	1.91 ± 0.97	1.05 ± 0.030	1.82	1.19
42-48	TALWITR (44)	2.84 ± 0.49	1.74 ± 0.31	1.63	1.07
49-55	IPQELVK (45)	2.69 ± 0.42	2.02 ± 0.32	1.33	0.85
57-66	GQFPIPSITW (6)	4.32 ± 0.51	0.72 ± 0.082	6	**3.92**
91-100	ELVVTGAYIK (7)	0.70 ± 0.082	0.14 ± 0.0071	5	**3.27**
138-151	EDEHPQCLNSQPHAR (46)	0.82 ± 0.11	0.77 ± 0.14	1.06	0.69
156-167	AIFSVGPVSPSR (47)	1.8 ± 0.21	1.15 ± 0.088	1.57	1.03
183-193	EWSLPS (48)	0.52 ± 0.11	0.33 ± 0.065	1.58	1.03
189-200	DLLELLVLGVSK (49)	2.92 ± 0.27	1.81 ± 0.017	1.61	1.05
192-200	ELLVLGVSK (50)	1.16 ± 0.21	0.84 ± 0.13	1.38	0.9
266-272	SYGGQYR (51)	0	0	-	-
291-299	DILIAGQFY (52)	0.89 ± 0.092	0.44 ± 0.066	2.02	1.32
354-372	YQAEFPMGPVTSAHAGTYR (53)	5.98 ± 0.90	3.35 ± 0.27	1.79	1.17
357-372	EFPMGPVTSAHAGTYR (54)	18.19 ± 2.27	15.01 ± 1.98	1.21	0.79

Epitope mapping was performed using hydroxyl radical foot-printing (HRF) and mass spectrometry techniques. Briefly, the ILT2-BND-22 or only ILT2 were exposed to hydroxyl radicals followed by digestion with Trypsin and AspN and liquid chromatography coupled with high-resolution mass spectrometry (LC-MS). The retrieved MS data comparing the protein and antibody-protein complex were used to map the epitope of the antibody. The rate constants for the modified peptides of ILT2 following trypsin digestion (Table 1S) or trypsin/Asp-N digestion (Table 2S) are presented. The highest normalized protection ratio (NR) values are bolded.