Supporting Information

Materials and Methods.

PBS buffer, DPBS buffer, DMEM, RPMI-1640 media and heat-inactivated fetal bovine serum were obtained from Corning-Mediatech. X-VIVO 15 serum-free media was purchased from Lonza. LB agar, 2xYT and Antibiotic-Antimycotic were purchased from Fisher Scientific and 4-12% Bis-Tris gels for SDS-PAGE were purchased from Bio-Rad. Heat-inactivated human male AB serum was purchased from Sigma-Aldrich. Human recombinant IL-2 was purchased from Biolegend. Absorbance spectra were measured with a SpectraMax i3x (Molecular Devices). Pierce High-Capacity Endotoxin Removal Spin Columns, Pierce LAL Chromogenic Endotoxin Quantitation Kit and LDH cytotoxicity assay kit were obtained from Thermo Fisher Scientific. Bicyclononyne-*N*-hydroxysuccinimide ester (BCN-NHS) and aminooxy-tetraethyleneglycol-azide (aminooxy-TEG-N_3) were purchased from Berry & Associates, Inc. 2'-(4-Methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MuNeuNAc) was obtained from Biosynth International Inc. All other chemicals were purchased from Sigma-Aldrich and used without further purification.

The following antibodies and recombinant proteins were used: Human recombinant Siglec-7-Fc chimera, Siglec-9-Fc chimera, NKG2D-Fc chimera proteins, AF488-labeled anti-Siglec-7 mAb (clone 194211) and blocking anti-NKG2D mAb (clone 149810) were purchased from R&D Systems. Fluorescein isothiocyanate (FITC)-labeled *Sambucus nigra* (SNA) lectin was obtained from EY Laboratories. AF647-labeled anti-HER2 mAb (clone 24D2), AF647-labeled anti-CD16 mAb (clone 3G8), AF647-labeled anti-CD56 mAb (clone HCD56), blocking anti-Siglec-7 mAb (clone S7.7), blocking anti-Siglec-9 mAb (clone K8) were obtained from Biolegend. TRITC-labeled anti-Fc mAb was purchased from Jackson Immunoresearch. FITC-labeled anti-CD3 mAb (clone BW264/56) was purchased from Miltenyi Biotec. Humanized trastuzumab with C-terminal aldehyde-tag was a gift from Catalent Pharma Solutions (Emeryville, CA).

Cell lines and cell culture. Breast cancer cells SKBR3, HCC-1954, MDA-MB-453, ZR-75-1, BT-20, MDA-MB-231, and MDA-MB-468 were obtained from American Type Culture Collection (ATCC).

SKBR3, HCC-1954, ZR-75-1, and MDA-MB-468 were maintained in RPMI-1640 media supplemented with 10 % heat-inactivated fetal bovine serum, plus 0.4 % Antibiotic-Antimycotic and L-glutamine (300 mg/L). MDA-MB-453, BT-20, and MDA-MB-231 were maintained in DMEM media supplemented with 10 % heat-inactivated fetal bovine serum, plus 0.4 % Antibiotic-Antimycotic, L-glucose (4.5 g/L), L-glutamine (584 mg/L) and sodium pyruvate (110 mg/L).

PBMCs were obtained from healthy blood bank donors and were isolated using Ficoll-Paque (GE Healthcare Life Sciences, GE-17-1440-02) density gradient separation. NK cells were isolated from PBMCs by negative selection using the MACS NK cell isolation kit (Miltenyi Biotec, 130-092-657) and LS columns (Miltenyi Biotec, 130-042-401) according to the manufacturer's protocol and cultured in X-VIVO 15 supplemented with 5 % heat-inactivated human male AB serum (Sigma-Aldrich), and 100 ng/mL recombinant human interleukin-2 (IL-2) (Biolegend) overnight before using. NK cell enrichment was verified by flow cytometry to result in > 95 % CD56+/CD3- cells (Fig. S3).

FACS analysis. Cells were incubated with sialidase, trastuzumab (Tras), trastuzumab-sialidase conjugate (T-Sia), or PBS control for 1 h at 37 °C. After three washes with PBS, cells were resuspended in cold PBS with 0.5% bovine serum albumin (BSA) containing the probe of choice: antibody, receptor-Fc fusion protein with secondary anti-Fc antibody pre-complexed in solution, or FITC-labeled SNA lectin. Cells and antibodies/fusion proteins were incubated for 30 mins at 4 °C in the dark. After three washes with PBS with 0.5% BSA, the cells were brought up in PBS with 0.5% BSA then analyzed by flow cytometry. All flow cytometry data was analyzed using FlowJo v. 10.0 (Tree Star).

Fluorescence Microscopy. For visualization of HER2-specific enzymatic activity of the conjugate: cells were incubated with various concentrations of T-Sia in PBS buffer for 1 hour at 37 °C. After washes with PBS, cells were then fixed with 4% formaldehyde at room temperature for 20 min. The fixed cells were washed with 0.5% BSA in PBS three times, followed by blocking in PBS with 0.5% BSA for 1 hour. Cells were incubated with FITC-labeled SNA (1:100) and AF647-labeled anti-HER2 antibody (1:100) in 0.5% BSA in PBS for 30 min at room temperature in the dark with gentle shaking. After washing thrice with 0.5% BSA in PBS, DAPI (1:1250 dilution from a 10 mM stock) was added right before imaging with a Nikon A1R+ Resonant Scanning Confocal Microscope.

For visualization of NK-tumor cell synapses: tumor cells were incubated with 6 nM Tras or 6 nM T-Sia in PBS buffer for 1 hour at 37 °C. Freshly isolated NK cells were added to tumor cells at an E/T ratio of 2:1 and incubated together for 15 min at 37 °C. After washing with PBS, cells were fixed with 4% formaldehyde in PBS for 20 min at room temperature. The fixed cells were washed with 0.5% BSA in PBS three times, followed by blocking in PBS with 0.5% BSA for 1 hour. Cells were incubated with a mixture of AF488-labeled anti-Siglec 7 (1:100), TRITC-labeled anti-Fc (1:400), and AF647-labeled anti-CD16 (1:100) antibodies in PBS buffer for 30 min at room temperature in the dark with gentle shaking. After washing thrice with 0.5% BSA in PBS, DAPI (1:1250 dilution from a 10 mM stock) was added right before imaging with a Nikon A1R+ Resonant Scanning Confocal Microscope.

Statistical analysis. Statistical analyses were conducted with Prism 6. Data are shown as mean ± SD of triplicate experiments, and significance was determined using a *t*-test, unless otherwise noted. **= $p < 0.005$, *= $p < 0.05$, and a p value > 0.05 was considered significant.

Table S1. Cytotoxic activity of isolated NK cells against various human breast cancer cells induced by Tras, a mixture of Tras and sialidase, or T-Sia. (N.D. none detected)

Cell line	HER2 level	EC_{50} (pM) / Maximal killing (%)		
		Tras	Tras/sialidase	T-Sia
SKBR3	+++	177±54 / 61±3	136±52 / 64±4	76±14 / 66±2
HCC-1954	+++	360±67 / 46±2	212±50 / 46±2	238±41 / 49±2
MDA-MB-453	++	110±27 / 71±3	77±17 / 78±3	22±5 / 75±2
ZR-75-1	+	1143±274 / 14±1	492±67 / 34±1	135±47 / 34±2
BT-20	+	1823±850 / 21±1	692±156 / 51±2	170±34 / 46±1
MDA-MB-231	+	N.D. / 6±1	N.D. / 10±1	N.D. / 10±1
MDA-MB-468	-	N.D. / 2±1	N.D. / N.D.	N.D. / 3±1

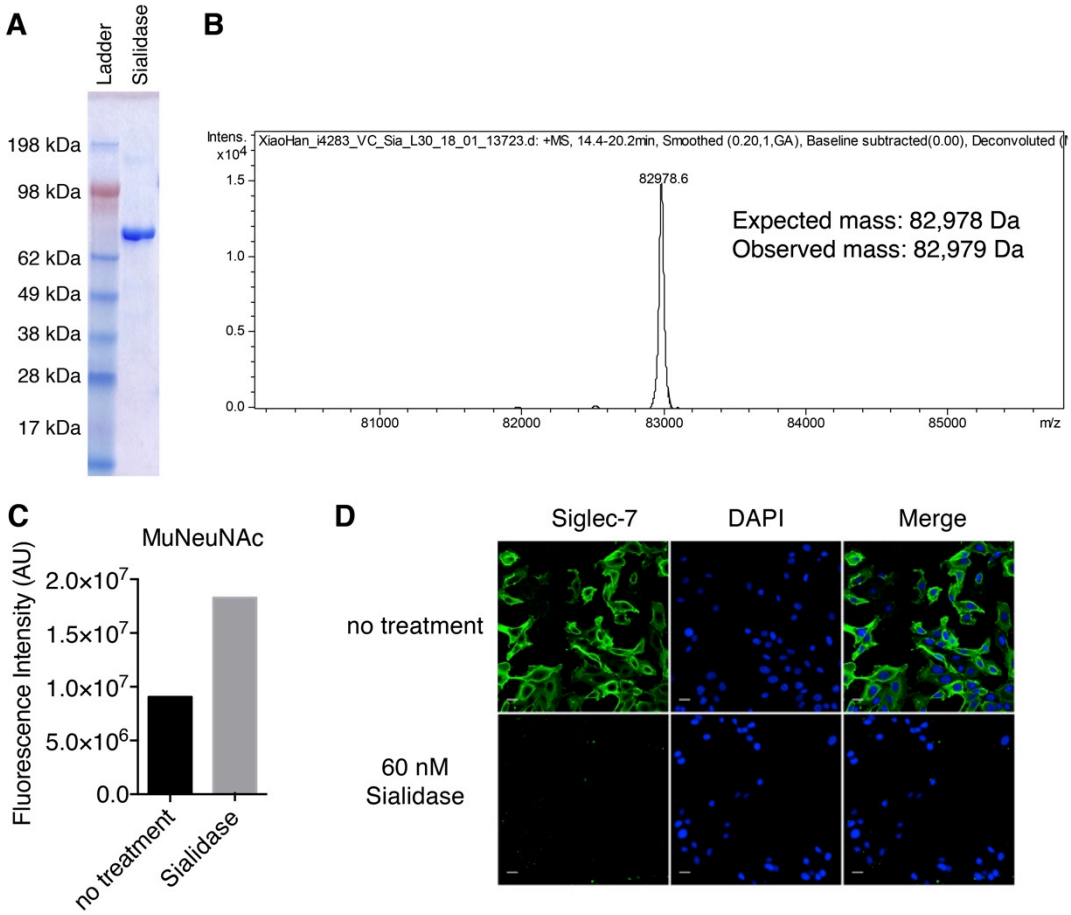

Figure S1. Purification and characterization of *Vibrio cholerae* sialidase. (A) Expression of wild-type *V. cholerae* sialidase analyzed by SDS-PAGE (stained with coomassie blue). (B) ESI-MS spectrum of purified *V. cholerae* sialidase. (C) Hydrolysis of 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MuNeuNAc) by *V. cholerae* sialidase. (D) Siglec-7 ligands on BT-20 cells are efficiently removed after treatment with *V. cholerae* sialidase. Scale bar = 25 μM.

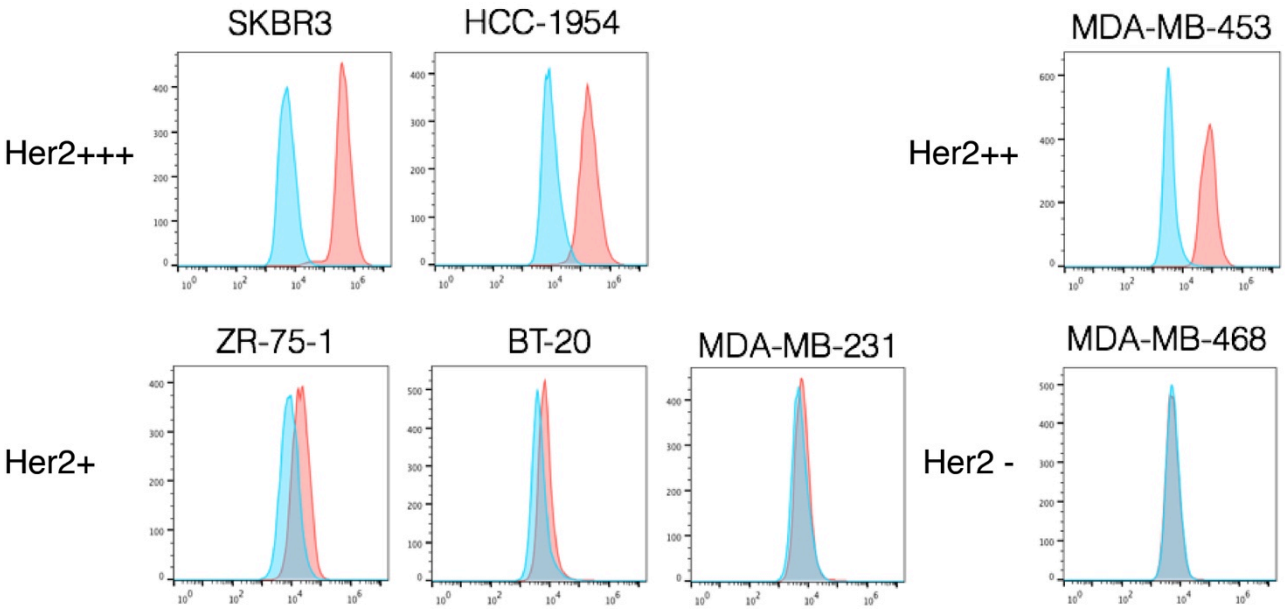

Figure S2. FACS analysis of HER2 expression in different breast cancer cell lines.

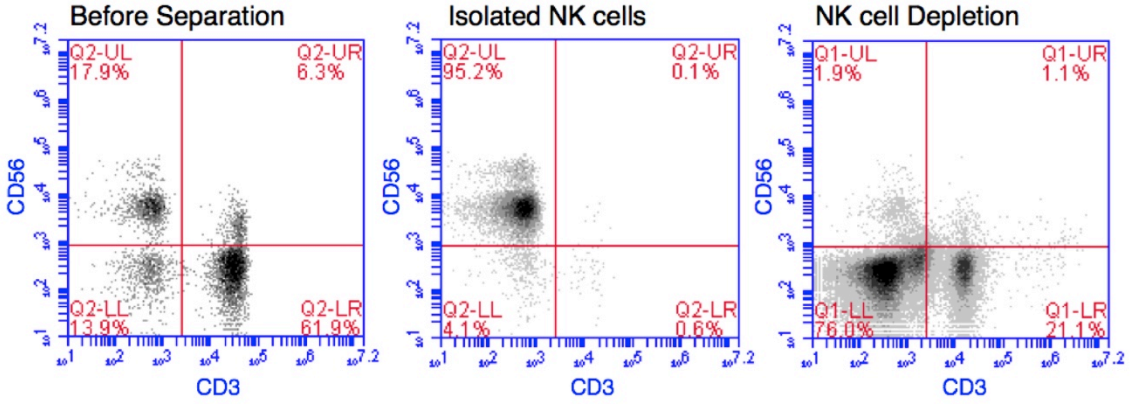

Figure S3. Flow cytometry and analysis of CD56 and CD3 markers on leukocytes. (A) Flow cytometry of PBMCs. (B) Flow cytometry of isolated NK cells. (C) Flow cytometry of PBMCs after NK cell depletion.

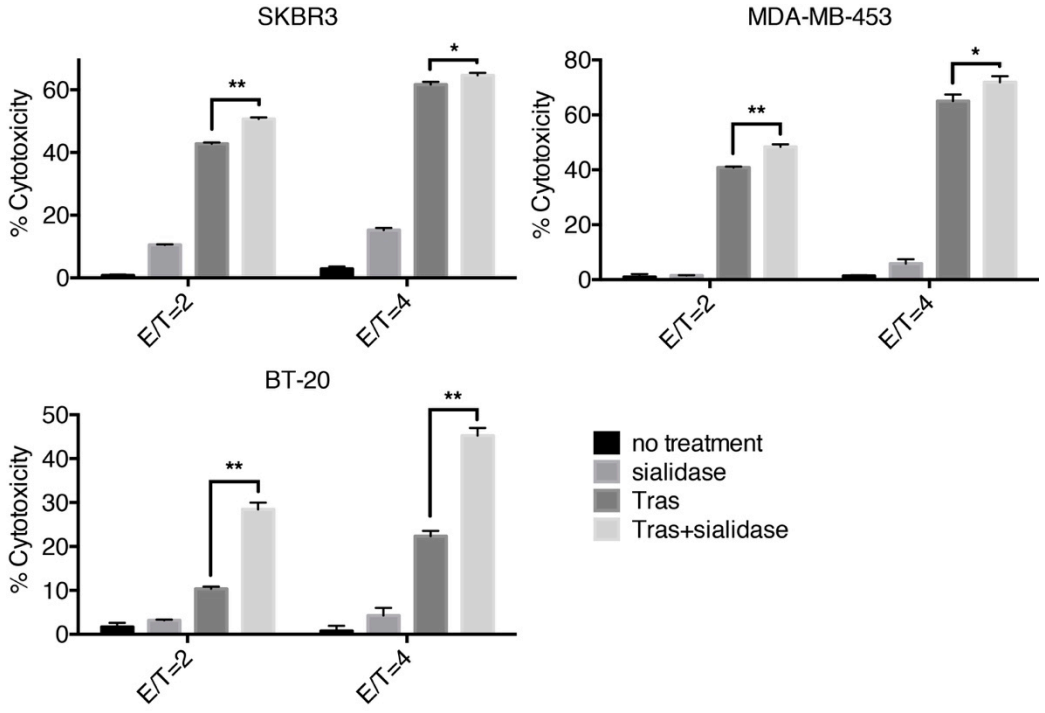

Figure S4: Cytotoxicity of isolated peripheral blood NK cells from healthy donors against different breast cancer cells in the absence or presence of free sialidase (30 nM), Tras (30 nM) or a mixture of free sialidase (30 nM) and Tras (30 nM) at E/T ratios of 2:1 and 4:1. *$P < 0.05$, **$P < 0.005$.

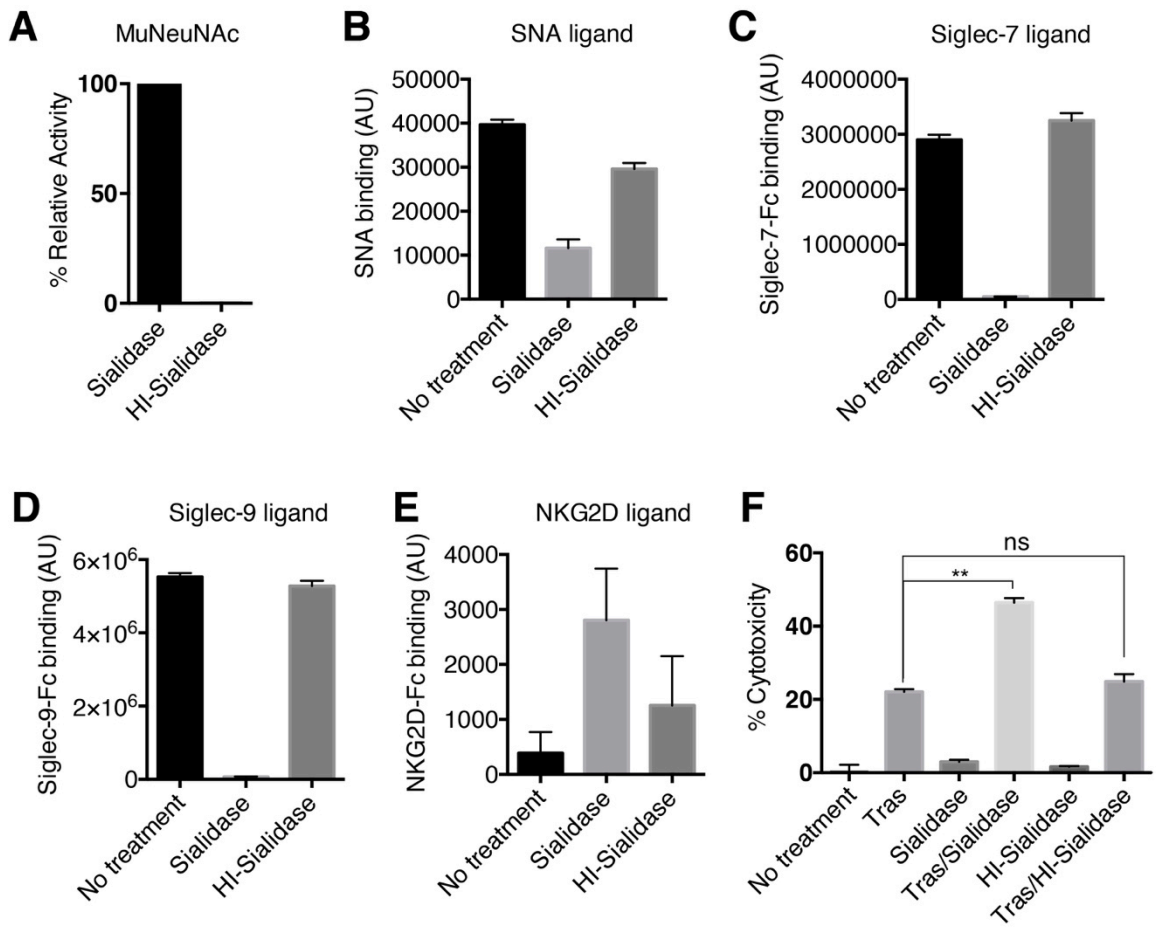

Figure S5. Characterization of wild-type and heat-inactivated *Vibrio cholerae* sialidase. (A) Hydrolytic activities of wild-type and heat-inactivated *V. cholerae* sialidase (HI-Sialidase) using 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MuNeuNAc). Sialidase was inactivated by incubation at 80 °C for 30 min. (B) Levels of *Sambucus nigra* lectin (SNA) ligands on BT-20 cells with or without 30 nM wild-type sialidase or heat-inactivated sialidase treatment. (C) Levels of Siglec-7 ligands on BT-20 cells with or without 30 nM wild-type sialidase or heat-inactivated sialidase treatment. (D) Levels of Siglec-9 ligands on BT-20 cells with or without 30 nM wild-type sialidase or heat-inactivated sialidase treatment. (E) Levels of NKG2D ligands on BT-20 cells with or without 30 nM wild-type sialidase or heat-inactivated sialidase treatment. (F) Cytotoxicity of isolated peripheral blood NK cells against BT-20 cells in the absence or presence of Tras (30 nM), sialidase (30 nM), a mixture of Tras (30 nM) and sialidase (30 nM), heat-inactivated sialidase (30 nM), or a mixture of Tras (30 nM) and heat-inactivated sialidase (30 nM) at an E/T ratio of 4:1. $^{**}P < 0.005$, ns: not significant.

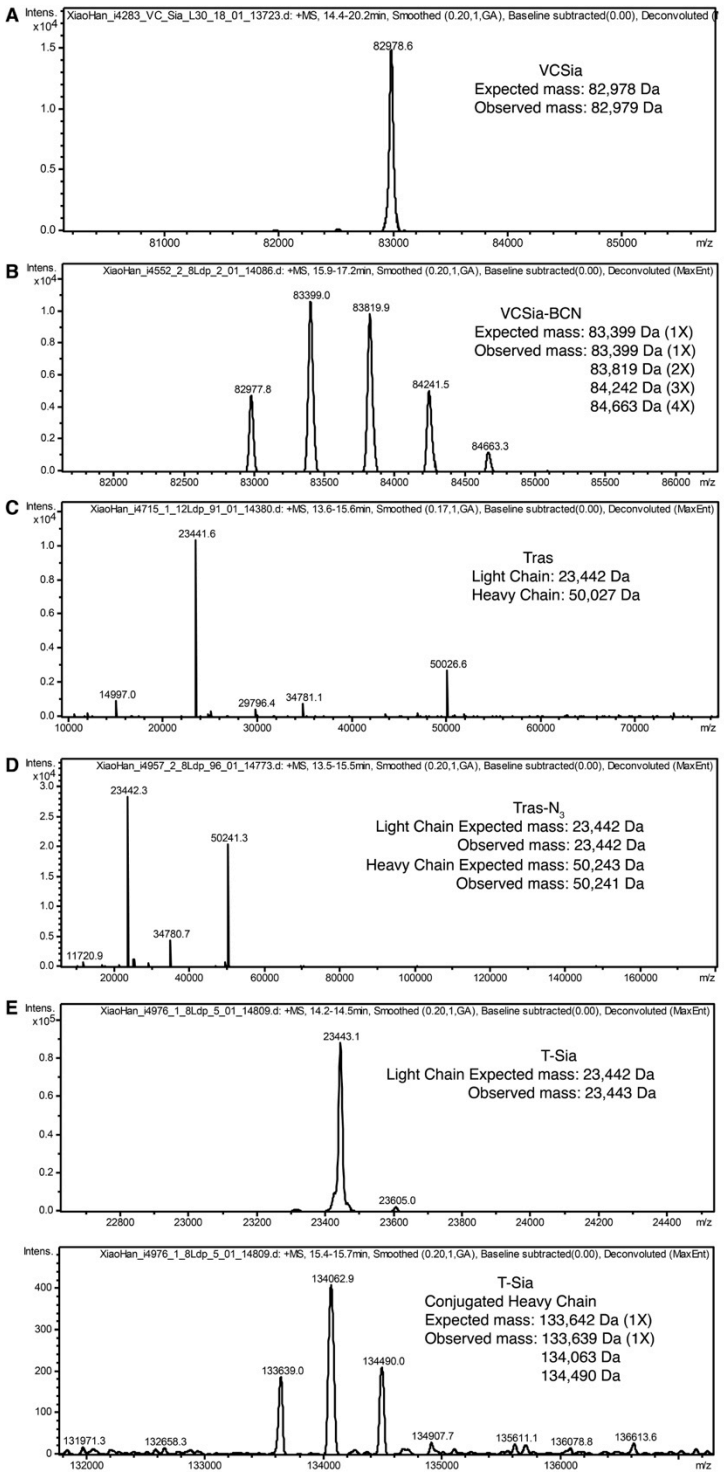

Figure S6. ESI-MS spectra of sialidase, Tras and its conjugates. (A) ESI-MS spectrum of purified *Vibrio cholerae* sialidase (VCSia). (B) ESI-MS spectrum of *V. cholerae* sialidase labeled with BCN-NHS (VCSia-BCN) at 1:12 molar ratio. (C) ESI-MS spectrum of Tras with C-terminal aldehyde

tag. (D) ESI-MS spectrum of Tras with C-terminal aldehyde tag conjugated with aminooxy-TEG-azide (Tras-N_3). (E) ESI-MS spectrum of T-Sia.

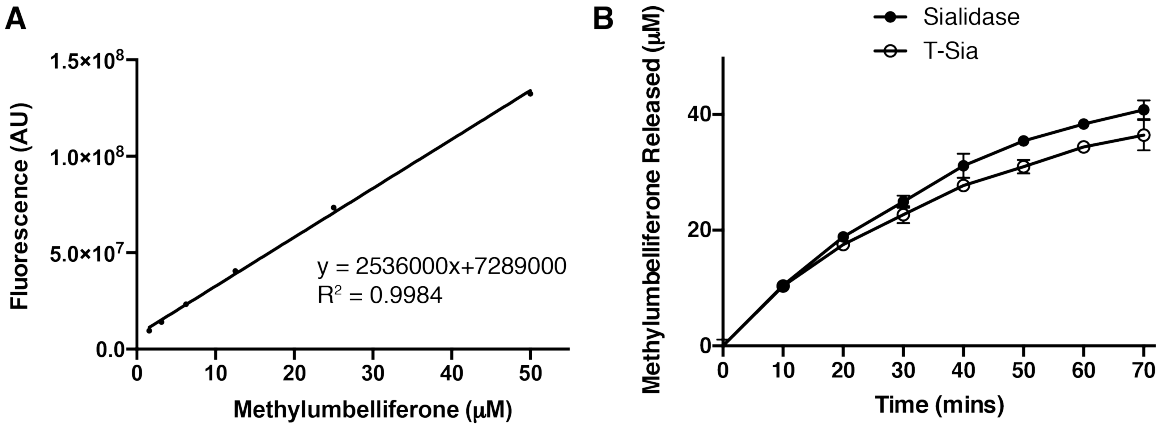

Figure S7. Hydrolytic activities of *V. cholerae* sialidase and T-Sia against fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MuNeuNAc). (A) Standard curve of methylumbelliferone concentration versus fluorescence. (B) Sialidase (1.2 nM) or T-Sia (0.6 nM) was incubated with 1 mM MuNeuNAc at 37 °C for various lengths of time. The methylumbelliferone released was measured with a fluorescence spectrophotometer (excitation 325 nm; emission 420 nm). Error bars represent standard deviation of triplicate samples.

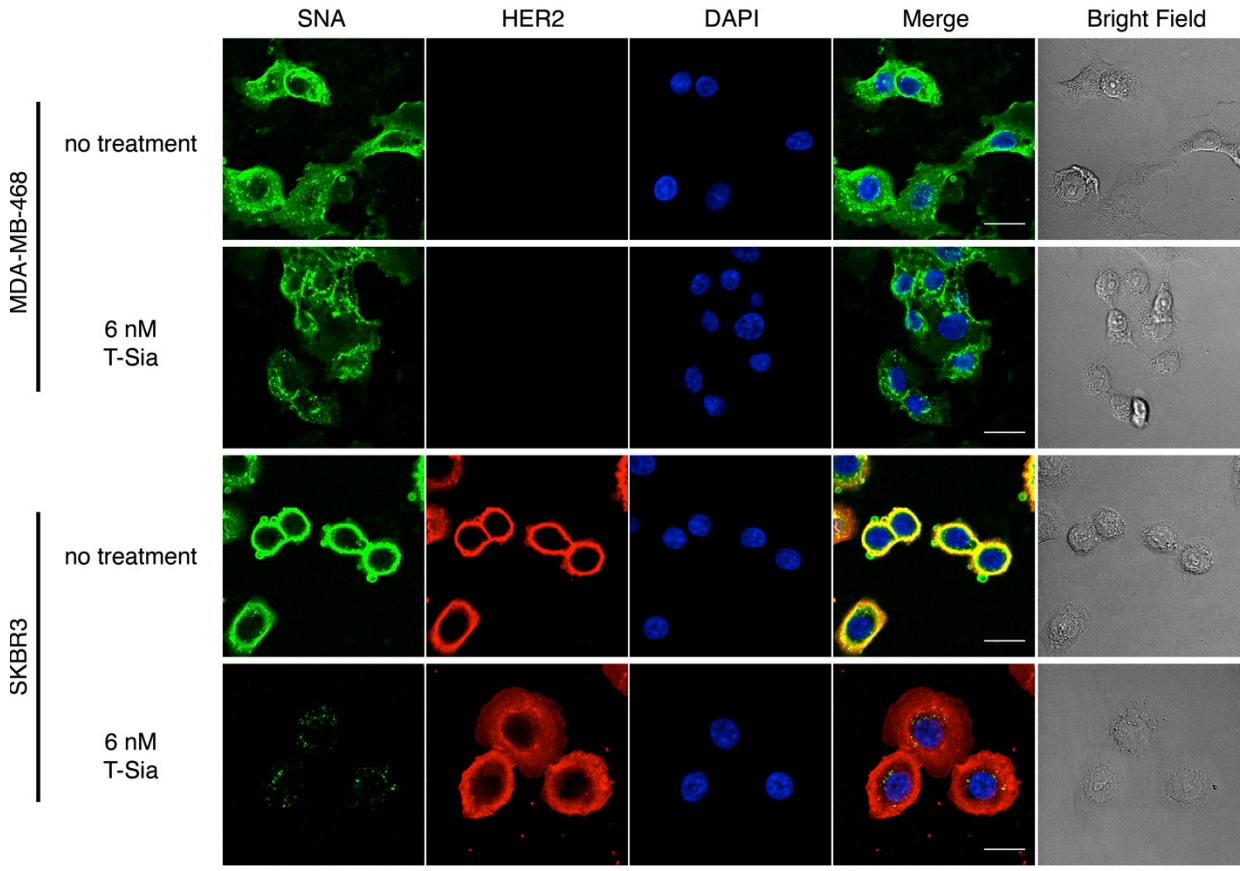

Figure S8. SNA ligands on individual cultures of SKBR3 and MDA-MB-468 cells in the absence or presence of T-Sia conjugate. Cells were incubated with 6 nM T-Sia conjugate or PBS in RPMI-1640 media for 1 hour at 37 °C and stained with FITC-labeled SNA lectin, AF647-labeled anti-HER2 and DAPI nuclear stain. Scale bar = 25 μM.

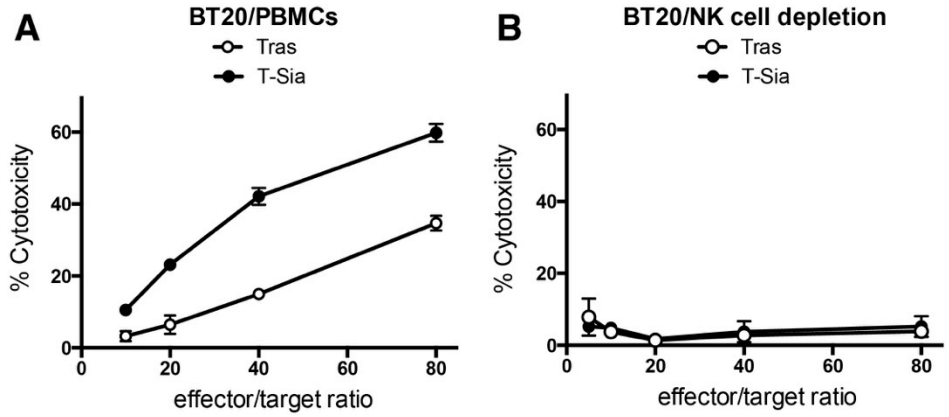

Figure S9. Cytotoxicity assays performed with BT-20 in the presence of Tras (30 nM) or T-Sia (30 nM) at various E/T ratios using PBMCs (A) or NK cell-depleted PBMCs (B).

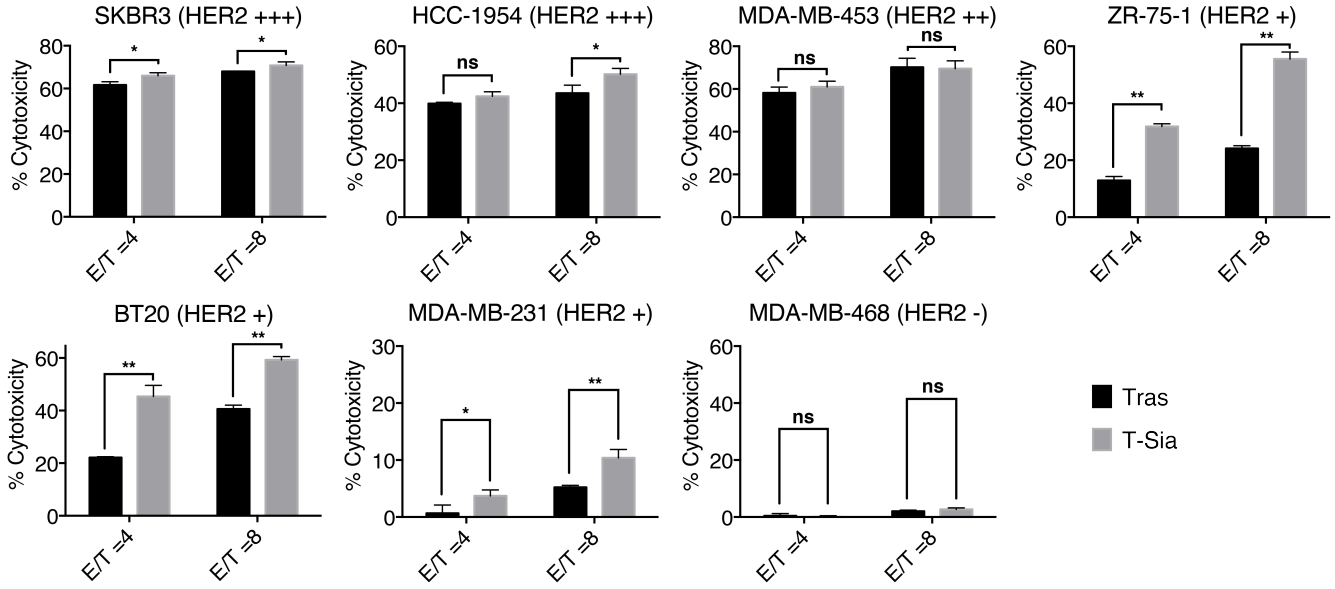

Figure S10. Cytotoxicity of isolated peripheral blood NK cells from healthy donors against different breast cancer cell lines in the presence of Tras (30 nM) or T-Sia (30 nM) at E/T ratios of 4:1 and 8:1. **P* < 0.05, ***P* < 0.005, ns: not significant.

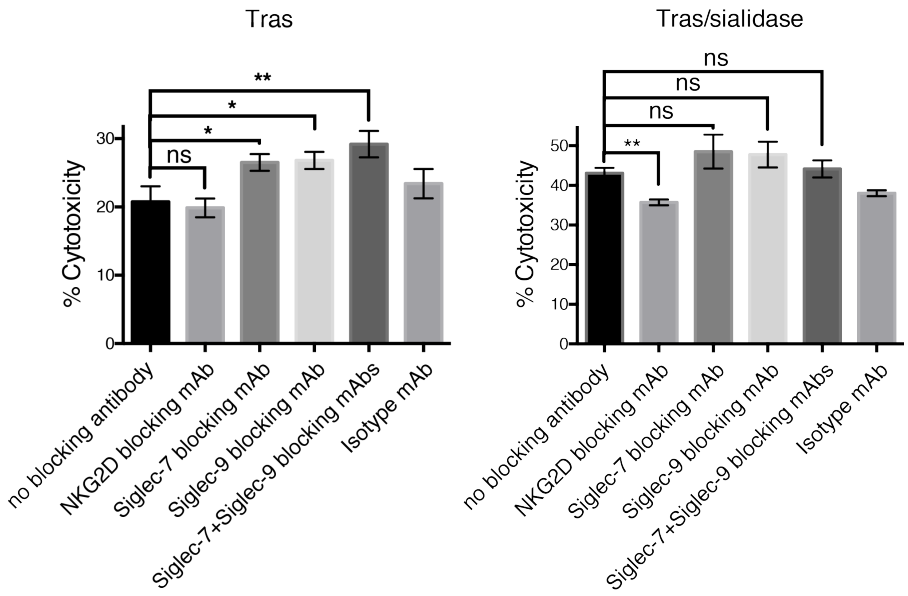

Figure S11. Cytotoxicity of isolated peripheral blood NK cells from healthy donors against BT-20 cells with Tras (30 nM) or a mixture of Tras (30 nM) and sialidase (30 nM) in the absence or presence of 5 μg/mL blocking anti-NKG2D (clone 149810), anti-Siglec-7 (clone S7.7), anti-Siglec-9 (clone S9), a mixture of anti-Siglec-7 (clone S7.7) and anti-Siglec-9 (clone S9), or mouse IgG1 isotype antibody (clone MOPC-21) at an E/T ratio of 4:1. **P* < 0.05, ***P* < 0.005, ns: not significant.