

Supplementary Information

A Single-step Chemoenzymatic Reaction for the Construction of Antibody-cell Conjugates

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➤ **General Considerations**

Materials and Reagents

Equipment

Cell culture

Mice

➤ **Supplementary Methods**

- 1 One-pot protocol for producing GDP-Fucose derivatives**
- 2 General procedure for enzymatic transfer of GDP-Fucose derivatives to cell surface**
- 3 Comparison of Sortagging and Fucosylation**
- 4 Preparation of GDP-Fucose modified antibodies**
- 5 General procedure for enzymatic transfer of GF-IgG to cell surface**
- 6 Primary human T cells preparation and IgG labeling on their surface**
- 7 IgG labeling on NK-92MI cells**
- 8 FACS and imaging analysis of binding between NK-92MI and BT474**
- 9 Analysis of NK-92MI cells-mediated cytotoxicity against HER2+ cancer cells**
- 10 Mice model of NK-92MI mediated killing of HER2+ cancer**
- 11 Primary OT-1 CD8+ T cells preparation and IgG labeling on their surface**
- 12 OT-1 T cells re-stimulation using OVA-pulsed splenocytes**
- 13 Analysis of OT-1 CD8+ T cells-mediated cytotoxicity against B16-OVA**

14 Statistical Analysis

➤ Supplementary Figures

S1 Chemical structures of GDP-Fucose derivatives including GF-Al-Biotin, GF-Al-Cy3, GF-Al-Tz and GF-Az-Tz.

S2 One-step enzymatic transfer of one-pot GDP-Fucose derivatives to Lec2 cells.

S3 One-step enzymatic labeling is more efficient and biocompatible than two-step.

S4 Direct comparison of FucT and Sortase A (5M) for NK-92MI cell surface engineering.

S5 Characterization of GDP-Fucose modified antibodies.

S6 Fluorescent SDS-PAGE gel analysis of IgG labeled cells.

S7 Flow cytometry analysis of the viability of Lec2 cells before and after IgG conjugation.

S8 Dual labeling of two different IgG molecules on Lec2 cells.

S9 GDP-Fucose or LacNAc analogues inhibit the FucT-mediated transfer of GF-IgG to the cell surface in a dose-dependent manner.

S10 Transfer of IgG to the NK-92MI cell surface enabled by additional fucosyltransferases and sialyltransferases..

S11 Transferring IgG molecules onto human T cells.

S12 Phenotype of human T cells before and after mIgG labeling.

S13 Transferring IgG molecules onto NK-92MI cells.

S14 Titration of GF-Herceptin concentrations in enzymatic transfer.

S15 Dual antibody labeling on NK-92MI cells.

S16 Proliferation and stability of Herceptin-NK-92MI conjugates with or without γ -irradiation.

S17 Herceptin-NK-92MI conjugate shows enhanced antitumor activity in established lung metastasis mice model.

S18 Enzymatic transfer of rIgG to OT-1 CD8⁺ T cells.

S19 Transferring α -PD-L1 onto OT-1 CD8⁺ T cells.

S20 Transferring α -PD-L1 onto OT-1 T cell surface enhance the cytokine secretion during killing cancer cells.

S21 Transferring α -PD-L1 onto T cell surface increase the size of T cell cluster and T cell proliferation during killing.

➤ Reference

General Considerations

Materials and Reagents

All chemical reagents and solvents were obtained from Sigma-Aldrich and used without further purification unless otherwise noted. All cell culture materials are listed in cell culture methods. ImmunoCult™ human CD3/CD28 T cell activator was purchased from STEMCELL Technologies, Inc. Recombinant mouse IFN- γ , recombinant mouse PD-L1-human Fc Chimera (PD-L1-hFc), CFSE cell division tracker kit, APC Streptavidin, anti-mouse CD3 (17A2)-FITC, anti-mouse CD8 (53-6.7)-PE, anti-mouse CD45.1 (A20)-Pacific blue, anti-mouse Thy1.1 (OX7)-Alexa Fluor 700, anti-mouse IgG (Poly4060)-APC, anti-human Fc (HP6017)-APC, anti-rat IgG (Poly4050)-APC, anti-His Tag (J095G46)-PE, anti-human CD3 (HIT3a)-APC, anti-human CD45 (HI30)-FITC, anti-human CD4 (RPA-T4)-PE/Cy7, anti-human CD8 (SK1)-Pacific Blue, anti-human CD25 (M-A251)-PerCP/Cy5.5, anti-human CD44 (BJ18)-FITC, anti-human CD45RO (UCHL1)-Alexa Fluor 700, anti-human CD62L (DREG-56)-PE and human Fc Receptor blocking solution were purchased from Biolegend. Bulky monoclonal antibodies including isotype mouse IgG2a (C1.18.4), anti-mouse PD-L1 (α -PD-L1, 10F.9G2), isotype rat IgG2b (LTF-2) and anti-human EGFR (528) were purchased from Bio X Cell. Therapeutic Herceptin were from Genentech. The control human IgG was obtained from Athens Research and Technology. Recombinant human HER2/ErbB2 Protein with His Tag (HER2-His) was purchased from Sino Biological, Inc. Click reagents including biotin-PEG3-azide (AZ-104, MW: 444.5), methyltetrazine-PEG4-azide (1014, MW: 389.40), methyltetrazine-PEG4-alkyne (1013-old, MW: 487.5, a discontinued product in their website), TCO-PEG4-NHS Ester (A137, MW: 514.6) and Cy5-TCO (1089, MW: 959.20) were purchased from Click Chemistry Tools LLC. Cy3-Azide (MW: 712.8) is a gift from Prof. Xing Chen's lab (PKU, China). (5OctdU)-5'-CAGTCAGTCAGTCAGTCAGT-3'(6-FAM) was ordered from Integrated DNA Technologies, Inc. D-Luciferin (monosodium salt), TNF- α mouse ELISA kit, IFN- γ mouse ELISA kit, Granzyme B human ELISA kit, DiD' solid, Hoechst 33342, DAPI, Alexa Fluor 647 NHS Ester, cell tracker green (CMFDA) and orange (CMTMR) were purchased from Thermo Fisher Scientific. 5 \times RIPA buffer kit were purchased from Cell

Biolabs, Inc. CytoTox 96® non-radioactive cytotoxicity assay and Bright-Glo™ luciferase assay system were purchased from Promega Corporation. Inorganic pyrophosphatase, FKP and FucT were expressed in endotoxin free bacteria (ClearColi® BL21-DE3, Lucigen) and purified as previously reported¹. Pure GDP-Fucose, GF-Al and BTTP were synthesized as previously described^{1,2}. The plasmid of Sortase A (5M) is a kind gift from Prof. Peng Chen (PKU). The enzyme was expressed and purified according to their work³. Human FucTs 1,3,5,6,7,8,9 were from Prof. Kelley Moremen lab (UGA). Free LacNAc was bought from Carbosynth (#OA08244). α 2,3 Sialyl LacNAc (sLacNAc) is a kind gift from Prof. Paulson (TSRI).

Equipment

All of the flow cytometry analyses were performed on an Attune NxT Flow Cytometer. Images of protein gels including coomassie SDS-PAGE gel and western blotting membrane were taken on ChemiDoc XRS+ (Bio-Rad). Absorbance, fluorescence intensity and luminescence were monitored in a Multi-Mode Microplate Reader (Synergy™ H4, Bio-Tek). Confocal images were taken on a Nikon spinning disk confocal microscope (TE2000). Fluorescent and phase contrast microscope images were taken on an All-In One fluorescence microscope (Keyence, BZ-X700). Bioluminescence imaging of live mice were acquired using an IVIS Spectrum system.

Cells

Cell lines were all purchased from ATCC unless otherwise specified. CHO cell lines (WT, Lec2 and Lec8, from Prof. Pamela Stanley at Albert Einstein College of Medicine) were grown as monolayer in alpha-Minimum Essential medium (α -MEM) (GIBCO) supplemented with 10% fetal bovine serum (FBS) (Omega Scientific, Inc). NK-92MI cell line was grown in MyeloCult™ H5100 (STEMCELL Technologies, Inc). Cancer cell lines including BT474, SKBR3, MDA-MB-435 (HER2+ and HER2-), MDA-MB-468, SKOV3 and mouse B16-OVA (from Prof. Gregoire Lauvau lab) are all grown in grown in DMEM (Dulbecco's modified Eagle's medium, GlutaMAX, GIBCO) supplemented with 10% FBS. Human blood samples were collected from healthy donors under the TSRI Normal Blood Donor Services program (#IRB 15-6710). Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll (Ficoll-Paque Plus, GE) density

centrifugation. PBMC, activated human T cells and mouse T cells were all cultured in RPMI 1640 (GlutaMAX) with 10% heat-inactivated FBS, 1 mM sodium pyruvate, 50 μ M β -ME, 10mM HEPES and 1 \times MEM NEAA (GIBCO) (referred as T cell culture media later). Cytokines in T cell culture media were added as indicated (rhIL2, rhIL7 and rhIL15 are all from NIH program). All cells cultures were incubated at 37 °C under 5% CO₂.

Mice

All mice were bred or housed under specific pathogen free (SPF) conditions. All animal experiments were approved by TSRI Animal Care and Use Committee. OT-1 mice are purchased from Taconic Biosciences. CD 45.1 mice from C57BL/6J (B6) genetic background were purchased from the Jackson Laboratory. The strain of OT-1+/-/CD 45.1+/- was generated by cross breeding. Six female B6 background P14 mice were gifts from Prof. John Teijaro lab. 176 female NSG mice are gifts from Prof. Philippe A. Gallay lab. Both male and female mice of 8–20 weeks of age were used for most experiments.

Supplementary Methods

1. One-pot protocol for producing GDP-Fucose derivatives

Reactions were typically carried out in a 15 mL corning tube with 5 mL 100 mM HEPES buffer (pH 7.5) containing L-fucose analogues (final concentration, 10 mM), ATP (10 mM), GTP (10 mM), MgSO₄ (10 mM), KCl (50 mM), inorganic pyrophosphatase (90 units, ~0.17 g/L, endotoxin free), and FKP (9 units, ~0.6 g/L, endotoxin free). The reaction mixture was incubated at 37 °C for 5–6 h with shaking (225 rpm). After the reaction finished (monitored by TLC analysis¹), enzymes were precipitated by adding 5 mL cold EtOH into the crude product. After the precipitates were removed through centrifuge (8000 \times g, 5 min), the crude products (containing ~10 mM GDP-Fucose analogues) could be directly used. For GF-AI and GF-Az, further modification could be achieved through CuACC reaction. Crude GF-AI/GF-Az sample (~5 mM, in HEPES buffer) were reacted with azide/alkyne probes (6 mM, the information of these commercial available compounds could be found in ***Materials and Reagents***) in the

presence of Cu/BTTP (1/2, 500 μ M) and sodium ascorbate (2 mM) at 30 °C for 6h (For Tz substrates, add one volume of MeOH in reaction mixture). After reaction finished (monitored by TLC and LC-MS analysis, representative figures of GF-Az-Tz and GF-Al-Tz are shown in **Spectra** part), BCS (bathocuproine sulphonate, 2 mM) were added to quench the reaction. These biocompatible crude products were lyophilized and reconstituted in pure water to a final concentration of 10 mM, which could be directly used in cell labeling. Following this protocol, we made one-pot products of GF-Al-Biotin, GF-Al-Cy3, GF-Al-Tz and GF-Az-Tz. Their structures are shown in **Figure S1**. Since GF-Al-Tz and GF-Az-Tz are important starting materials for making GF-IgG, we purified these two GDP-Fucose derivatives and characterize them using high-resolution ESI-TOF MS and NMR (**Spectra**), which also confirm the one-pot procedure is reliable.

2. General procedure for enzymatic transfer of GDP-Fucose derivatives to cell surface

Live cells (1~2 million) were resuspended in 100 μ L HBSS buffer containing 20 mM MgSO₄, 3 mM HEPES, 0.5% FBS, 100 μ M GDP-Fucose derivatives and 30 mU FucT (~0.02 mg/mL). After the incubation for 20 minutes (works from on ice to 37 °C), the cells were washed with PBS and ready for further application or analysis. For biotin detection, the cells were stained with APC Streptavidin after reaction. For Tz detection, the cells were reacted with 20 μ M TCO-Cy5 on ice for 30 minutes and washed three times.

3. Comparison of Sortagging and Fucosylation

For sortagging reaction, NK-92MI cells (5 million/mL) were incubated with soluble SrtA and biotin-LPETG in RPMI complete media (10% inactivated fetal bovine serum, 1% MEM (Gibco) Non-Essential Amino Acids Solution, 1 mM sodium pyruvate, 1% GlutaMAX, (Gibco) 0.1 mM β -mercaptoethanol) supplemented with 10 mM CaCl₂ at 37 °C for 2 hr⁴. For fucosylation reaction, NK-92MI cells (5 million/mL) were incubated with FucT and GF-biotin in fucosylation buffer described in Methods 2 for different times (2-30 min). Different concentrations of enzymes and substrates were used as indicated.

4. Preparation of GDP-Fucose modified antibodies

All of the antibodies (full-length IgG, MW: ~150 KDa) for conjugation were first desalted into PBS and concentrated to a 6 mg/mL solution. TCO group was first introduced onto antibodies according to the standard labeling protocol of TCO-PEG4-NHS ester (<https://clickchemistrytools.com/product/tco-peg4-nhs-ester/>) and previous reports^{5,6} about its application on IgG labeling. Briefly, we prepared fresh 50 mM stock of TCO-PEG4-NHS reagent in DMSO and add it to the IgG sample (final concentration 6 mg/mL) at a final concentration of 0.5 mM. The reactions were incubated at room temperature for 30 minutes and quenched by adding Tris buffer (pH 8.0) to a final concentration of 50mM Tris. The quenched reaction mixtures were incubated at room temperature for 5 minutes and then desalted into PBS using G25 desalting column (PD-10, GE). The concentrations of desalted TCO-IgGs were around 4.5 mg/mL (~1-4 TCO per IgG). After that, ~10 mM one-pot products of GF-Az-Tz were added to TCO-IgGs with a final concentration at 0.15 mM (5eq of IgG). After 30 minutes of incubation at room temperature, these one-pot GF-IgG products were ready to use and could be kept at 4 °C for up to 2 months. All the GDP-Fucose modified antibodies were characterized by MALDI-TOF MS. The molecular weights and average numbers of GF modification were shown in **Supplementary Figure S5B**. To make Alexa Fluor 647 labeled GF-IgGs used in confocal imaging and fluorescent gel analysis, the GF-IgG molecule was labeled with Alexa Fluor 647 probes following the manual. The antigen binding affinities of GF-Herceptin, GF-anti-EGFR and GF-anti-PD-L1 were titrated on MDA-MB-435 Her2, SKOV3 and B16-OVA (IFN- γ treated) cells, respectively, according to the reference⁵.

5. General procedure for enzymatic transfer of GF-IgG to cell surface

Live cells (1~2 million) were resuspended in 100 μ L HBSS buffer containing 20 mM MgSO₄, 3 mM HEPES, 0.5% FBS, 0.1 mg/mL GF-IgG (different concentrations were used in titration) and 60 mU FucT (~0.04 mg/mL) (additional MgSO₄ is not needed if use RPMI 1640 as reaction buffer). After the incubation for 20 minutes (on ice, room temperature or 37 °C), the cells were washed with PBS twice and ready for further application or analysis. **For flow cytometry analysis**, IgG labeled cells were stained with

DAPI and fluorescent secondary antibody against labeled IgG (1/50-1/200 dilution) on ice for 30 minutes. **For confocal imaging**, Lec2 cells plated on a chamber cover glass (Nunc) were treated with 0.1 mg/mL Alexa Fluor 647 labeled GF-rIgG in the same fucosylation condition as described above. After conjugation, live cells were washed and stained with Hoechst 33342 for 30 minutes on ice and then washed for imaging. **To confirm the binding activity of cell surface conjugated antibodies**, labeled cells were allowed to bind with 10 µg/mL antigen (PD-L1-hFc or HER2-His) in binding buffer (HBSS with 5mM HEPES, 2mM CaCl₂, and 1mM MgCl₂) on ice for 30 minutes. After binding, cells were washed twice with PBS and stained with DAPI and APC-anti-human Fc (1/50 dilution) or PE-anti His (1/100 dilution). **For the SDS-PAGE fluorescent gel imaging analysis**, fluorescent GF-IgG-A647 was used instead of GF-IgG. At the saturated labeling condition, labeled cells and control cells were collected and washed three times. After that, counted cells were lysed in SDS loading buffer and subjected to SDS-PAGE analysis. Pure GF-IgG-A647 proteins were also loaded as standards in quantification. For example, 2×10^5 Lec2 cells labeled with IgG-A647 were lysed in 20 µL SDS loading buffer and loaded to one lane of a SDS-PAGE gel. In the same gel, 1 ng, 5ng and 10ng pure GF-IgG-A647 proteins were loaded on separated lanes as the references. The resolved fluorescent gel was analyzed by ChemiDoc XRS+ (Bio-Rad). In brief, a linear plot was generated from the reference protein intensities and the quantity of IgG labeled on 2×10^5 Lec2 cells was then derived by cross-referencing its intensity, which is 12.6 ng. As the MW of IgG is around 150KDa, we can calculate the total IgG numbers labeled on 2×10^5 Lec2 cells were $(12.6 \times 10^{-9} / 150,000) \times 6.02 \times 10^{23} = 5 \times 10^{10}$. Then, each Lec2 cell has $(5 \times 10^{10}) / (2 \times 10^5) = 2.5 \times 10^5$ IgG molecules.

6. Primary human T cells preparation and IgG labeling on their surface

Fresh PBMCs were freshly prepared as described above. 4 million per mL PBMCs were cultured in T cell culture media with 15 ng/mL rhIL2 and activated with human CD3/CD28 T cell activator for two days. After that, activated human T cells were kept under 4×10^6 cells/mL in T cell culture media (fresh media with cytokine were added every two days). Phenotypes were characterized after two weeks expansion (>95% are human T cells). LacNAc levels on CD4+ and CD8+ T cells were tracked through

fucosylation with GF-biotin on day 0 (naïve T cells), day 2, day 4, day 7, day 11 and day 13. Activated human T cells were labeled with mIgG using the general procedure for enzymatic GF-IgG transfer. After that, the labeling detection was confirmed. Labeled human T cells were then cultured in T cell culture media at a start cell density of 5×10^5 cells/mL. The decay of cell surface mIgG molecule was tracked in 24 hours after labeling (anti-mouse IgG staining). The cell proliferation rate of labeled T cells was compared with unlabeled human T cells in three days (live cell counting).

7. IgG labeling on NK-92MI cells

NK-92MI cells or irradiated NK-92MI cells (6 Gy) were labeled with Herceptin or control human IgG according to the general protocol. After labeling, the labeling detection and the antigen (HER2-His) binding were both confirmed. NK-92MI cells with or without IgG conjugation were then cultured in T cell media with a start cell density of 5×10^5 cells/mL. The decay of cell surface conjugated Herceptin were tracked at day 0, day 1 and day 2 (anti-human Fc staining). The proliferation rates of cells were tracked at day 0, day 1, day 2 and day 3 (DAPI staining and FACS counting). For dual antibodies labeling, NK-92MI cells were conjugated with GF- α -EGFR first and then conjugated with GF-Herceptin after washing.

8. FACS and imaging analysis of binding between NK-92MI and BT474

For flow cytometry analysis, NK-92MI cells were stained with CFSE and then labeled with Herceptin or not according to the general protocol. BT474 cells were stained with DiD first and then mixed with Herceptin labeled or unlabeled NK-92MI cells at the ratio of 1:1. Two hours later, the cells mixture was analyzed by flow cytometry. For fluorescence imaging, BT474 cells were stained with CFSE and cultured in glass bottom petri dish overnight. NK-92MI cells were then stained with cell tracker orange and then labeled with Herceptin or not. NK-92MI cells were added to BT474 culture at ratio of 1:1. Two hours later, the co-cultured cells were imaged by fluorescent microscope before and after PBS wash.

9. Analysis of NK-92MI cells-mediated cytotoxicity against HER2+ cancer cells

Labeled or unlabeled NK-92MI cells were co-cultured with different type of cancer cells at indicated effector/target ratios for 4 hours in a 96-well plate. In most of the experiments, the effector/target ratio is 5/1. Free Herceptin were added at a final concentration of 5 µg/mL if indicated. Specific cancer cell lysis was detected by LDH secretion in supernatant (CytoTox 96, Promega). Set-up of control groups and calculations of specific lysis were according to manufactory's instruction (https://www.promega.com/products/cell-health-assays/cell-viability-and-cytotoxicity-assays/cytotox-96-non_radioactive-cytotoxicity-assay/?catNum=G1780). Supernatant of each group (one hour incubation) were also collected and subjected to granzyme B ELISA kit for quantification.

10. Mice model of NK-92MI mediated killing of HER2+ cancer

Thirty female NSG mice (6-8 weeks old) were inoculated with 5×10^5 MDA-MB-435 HER2+/F-luc cells through tail vein injection. One day later, mice were randomly divided into three groups (10 mice per group). Each group were treated with HBSS, NK-92MI or Herceptin labeled NK-92MI cells through tail vein injection (3×10^6 NK cells each mice). Six days after tumor challenge, mice were injected with 200 µL D-luciferin (15mg/mL) through *i.p.* injection. Twelve minutes later, the bioluminescence signal in mice were analyzed by PerkinElmer IVIS system. The total photons indicating the tumor mice were quantified by IVIS software. For the established tumor model, NSG mice were injected intravenously with 5×10^5 MDA-MB-435/HER2+/F-luc cells on day 0. Three days later, the animals were imaged to confirm the tumor formation and then treated by *i.v.* injection of 5×10^6 NK-92MI or Herceptin-NK-92MI cells or HBSS. The animals were imaged again on day 4. The animals were received one more treatment of 5×10^6 NK-92MI or Herceptin-NK-92MI cells (*i.v.* injection) on day 9 and day 17. On day 23, all of the mice were sacrificed and the lungs were collect for bioluminescence analysis.

11. Primary OT-1 CD8+ T cells preparation and IgG labeling on their surface

Splenocytes from OT-1 mice were activated by 1nM OVA peptides in T cell media for two days. After that, activated cells were *in vitro* expanded in fresh T cell media with 15 ng/mL rhIL2 or 10 ng/mL rhIL7 and 20 ng/mL rhIL15 for several days (kept under 8×10^6

cells/mL, fresh media with cytokine were added every two days). Phenotypes were characterized (>95% are OT-1 CD8⁺ T cells). LacNAc levels on OT-1 T cells (cultured with two different cytokines) were tracked through fucosylation with GF-biotin on day 0 (naïve T cells), day 2, day 4, day 7, day 9, day 11 and day 13. Activated OT-1 T cells were labeled with α -PD-L1 and mIgG control using the general procedure for enzymatic GF-IgG transfer. After that, the labeling detection and the antigen (PD-L1-hFc) binding were both confirmed. Labeled OT-1 T cells were then cultured in T cell culture media at a start cell density of 5×10^5 cells/mL. The decay of cell surface α -PD-L1 molecule was tracked in 24 hours after labeling (anti-rat IgG staining).

12. OT-1 T cells re-stimulation using OVA-pulsed splenocytes

Splenocytes from B6 WT mice were pulsed with SIINFEKL (OVA) peptide (1 μ g/mL, in T cell media) for 2h. After that, cells were washed three times before use. OT-1 T cells with CD45.1 congenic marker were stained with CFSE and then labeled with rIgG. 10^5 OT-1 T cells (labeled or unlabeled) were mixed with 10^6 OVA-pulsed splenocytes in 500 μ L T cell media. Control groups were also set up without splenocytes. The cell mixtures were cultured for 3 days. After that, cells were stained with APC anti-CD45.1 and DAPI. The CFSE dilution signals were analyzed on live CD45.1 positive cells.

13. Analysis of OT-1 CD8⁺ T cells mediated cytotoxicity against B16-OVA

B16-OVA cells (stably transduced with firefly luciferase) were seeded in 96-well plate and treated with 10 ng/mL IFN- γ overnight (B16-OVA cells were all treated with IFN- γ to induce high expression level of PD-1 in this work). Labeled or unlabeled OT-1 cells were co-cultured with B16-OVA cancer cells at indicated effector/target ratios for 3 hours or 20 hours in a 96-well plate (free anti-PD-L1 was added at 5 μ g/ml when indicated). In most of the experiments, the effector/target ratio is 5/1. Phenotype of T cell clusters was imaged before cell number quantification. B16-OVA cell numbers were quantified through the luciferase activity according to the reference. The detection reagent was directly added to medium in each well according to the manufactory's manual (Bright-Glo, Promega). For cytokine secretion quantification, cells were cultured for 9 hours and supernatant were collected and subjected to TNF- α and IFN- γ ELISA kit.

For OT-1 T cells proliferation in killing B16-OVA, OT-1 cells were stained by CFSE before IgG labeling. Modified cells were mixed with B16-OVA cells in the effector/target ratio of 2/1. After 72 hours, the cell mixtures were stained with APC-anti CD8 and DAPI. CFSE dilution signal was analyzed on CD8+ cells.

14. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 7.0). Comparisons over groups were analyzed using two-way ANOVA tests followed by Sidak's multiple comparisons test, and comparisons of multiple samples at one group were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. For cell-based experiments, three biological replicates were performed. For tumor experiments in NSG mice, each group has 10 mice. In all figures, ns, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

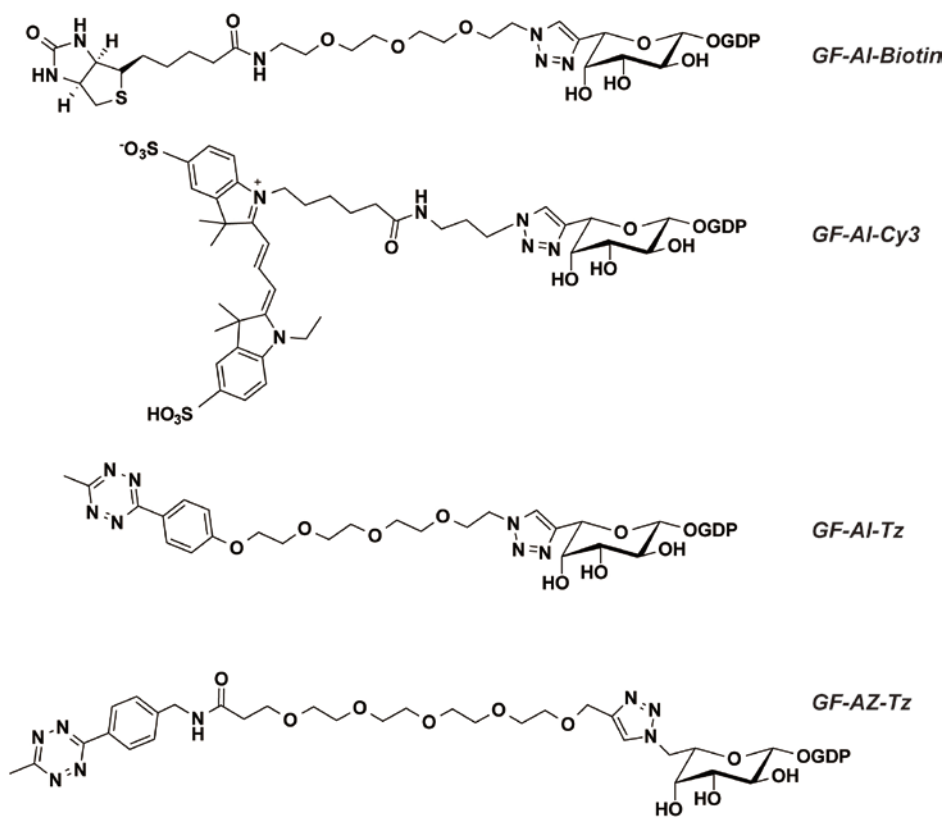


Figure S1. Chemical structures of GDP-Fucose derivatives including GF-Al-Biotin, GF-Al-Cy3, GF-Al-Tz and GF-Az-Tz.

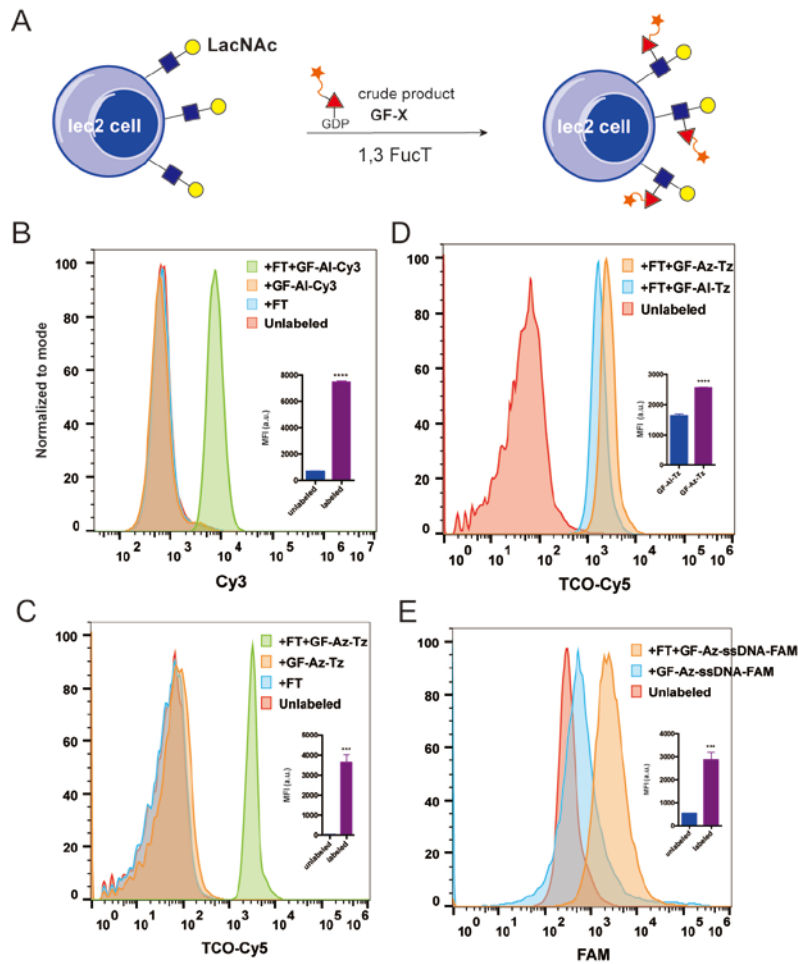


Figure S2. The one-step enzymatic transfer of one-pot GDP-Fucose derivatives to Lec2 cells. (A) Schematic representation of transferring one-pot GDP-Fucose derivatives using FucT to LacNAc epitopes on Lec2 CHO cell surface. (B) Lec2 cells were treated with FucT and GF-Al-Cy3, FucT alone, or GF-Al-Cy3 alone, or untreated. Then cells were analyzed by flow cytometry, which only showed successful labeling when treated with both FucT and GF-Al-Cy3. (C) Lec2 cells were treated with FucT and GF-Az-Tz, FucT alone, or GF-Az-Tz alone, or untreated. Then the cells were reacted with TCO-Cy5 and analyzed by flow cytometry. Significant labeling was only observed in the group treated with FucT and GF-Az-Tz. (D) GF-Al-Tz and GF-Az-Tz were compared in the FucT-mediated transfer reaction on Lec2 cells. Cells were reacted with TCO-Cy5 after enzymatic reaction and analyzed by flow cytometry. The GF-Az-Tz group generated stronger signal than GF-Al-Tz. (E) Lec2 cells were treated with FucT and GF-Az-ssDNA-FAM, or GF-Az-ssDNA-FAM alone, or untreated. Then the cells were analyzed

by flow cytometry. The group treated with FucT and GF-Az-ssDNA-FAM has the strongest FAM signal. Error bars, mean values \pm SD. ****P < 0.0001; one-way ANOVA followed by Tukey's multiple comparisons test.

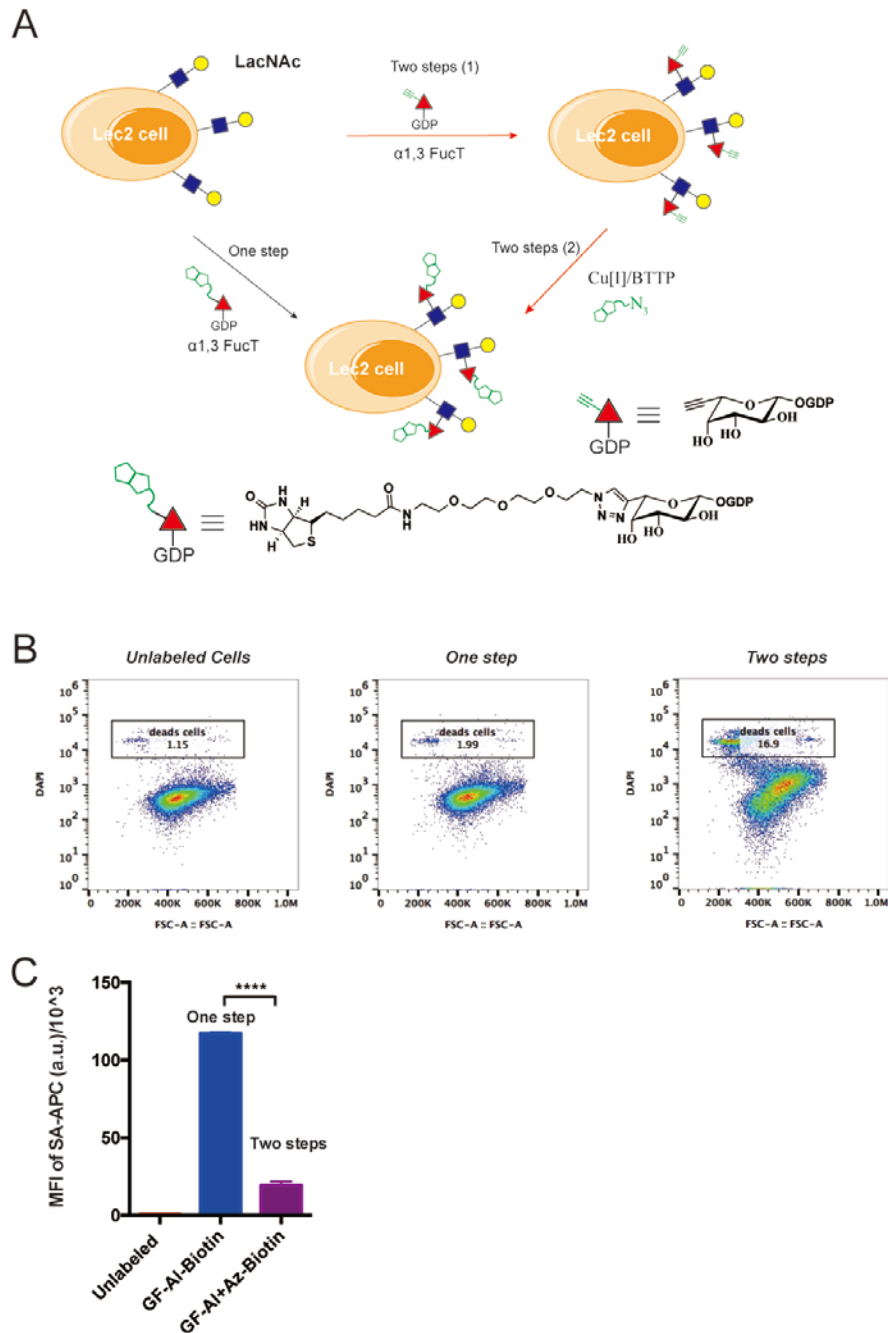


Figure S3. The one-step enzymatic labeling is more efficient and biocompatible than the two-step approach. (A) Schematic representation of the one-step and the two-step labeling systems. In the one step labeling system, the one-pot product of GF-AI-biotin

was directly transferred to LacNAc epitope on Lec2 cell surface using FucT. By contrast, the two-step labeling involved the first step of enzymatic transfer of GF-Al and a followed step of CuAAC reaction between surface alkyne and azide-biotin probe. **(B)** Flow cytometry analysis of biocompatibility in one-step or two-step labeling process. Lec2 cells after reaction were stained with DAPI. **(C)** Comparison of efficiency in one-step and two-step labeling process described in (A). Cells were stained by streptavidin-APC. Error bars, mean values \pm SD. ****P < 0.0001; one-way ANOVA followed by Tukey's multiple comparisons test.

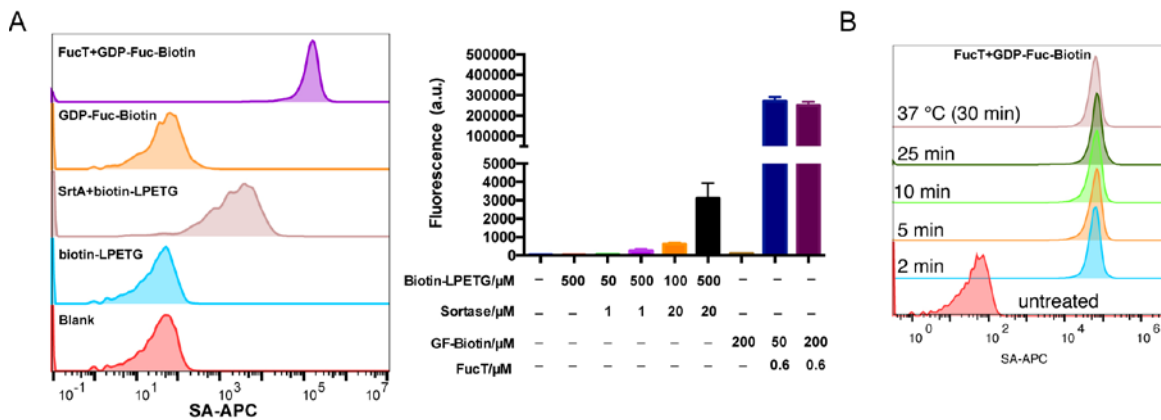
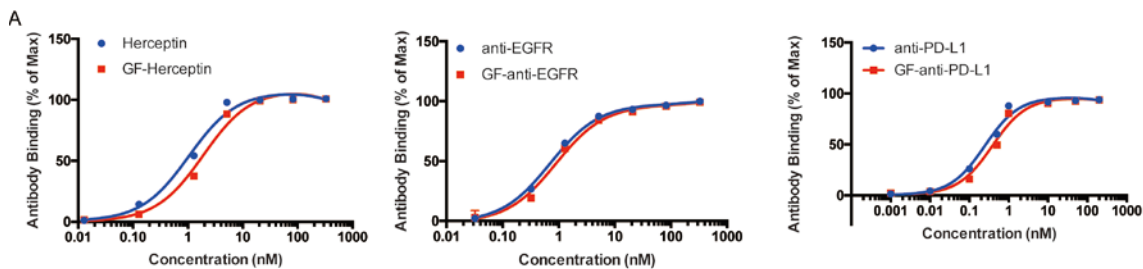


Figure S4. Direct comparison of FucT and Sortase A (5M) for NK-92MI cell surface engineering. **(A)** FucT and GF-biotin were used in the cell-surface fucosylation and SrtA 5M and biotin-LPETG were used in sortagging. Enzyme alone, biotin probe alone or untreated were shown as negative controls. Different enzyme and probe concentrations were used in the direct comparison experiment. **(B)** Time course of enzymatic transfer of GF-biotin to NK-92 MI cells on ice; reaction at 37 °C was used as the maximum labeling control.



B

	Ab		GF-Ab		aver. numbers of GF/Ab
	[M+H] ⁺	[M+H] ²⁺	[M+H] ⁺	[M+H] ²⁺	
Herceptin	148876	74495	154414	77511	3.7
human IgG	148822	74472	153514	77124	3.2
anti-PD-L1	147952	74092	149639	74921	1.1
anti-EGFR	150681	75342	152294	76353	1.1
rat IgG	150596	75330	151989	76118	1
mouse IgG	148275	74082	150319	75377	1.4

Figure S5. Characterization of GDP-Fucose modified antibodies. (A) Antigen binding capacities of GDP-Fucose modified antibodies were titrated and compared with unmodified antibodies. (B) Molecular weights of GDP-Fucose modified antibodies were characterized by MALDI-TOF MS. Average numbers of GDP-Fucose on each antibody were calculated (one GF attachment leads to a 1489 Da shift).

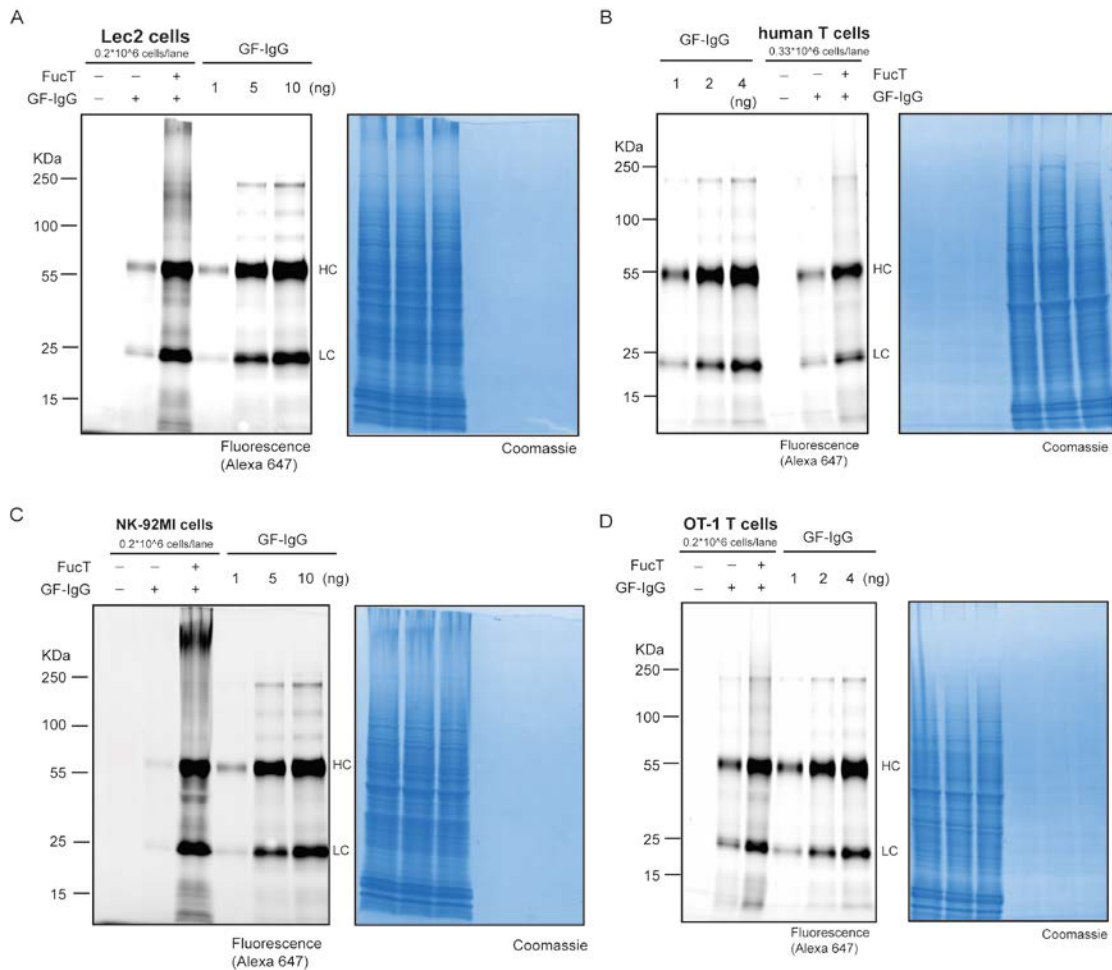


Figure S6. Fluorescent SDS-PAGE gel analysis of IgG labeled cells. GF-IgG molecules that are modified with Alexa Fluor 647 on the IgG portions were used to quantify the numbers of IgG molecules conjugated to the surface of one cell. Cells were washed three times after treated with antibody and FucT. Different amounts of pure GF-IgG-A647 proteins were used as standards in the quantification. At the saturated condition, approximately 2.5×10^5 IgG molecules were introduced to one Lec2 cell surface (A); approximately 2.9×10^4 IgG molecules were introduced to one primary human T cell surface (B); approximately 3×10^5 IgG molecules were introduced to one NK-92MI cell surface (C); approximately 7×10^4 IgG molecules were introduced to one mouse OT-1 T cell surface (D). The example of IgG numbers calculation could be found in Supplementary Methods 5.

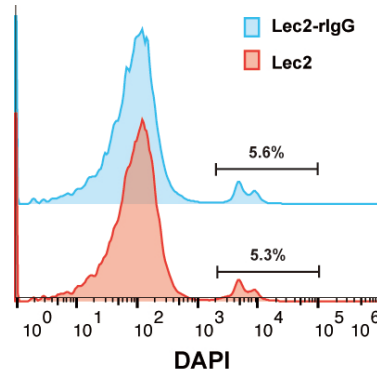


Figure S7. Flow cytometry analysis of the viability of Lec2 cells before and after IgG conjugation.

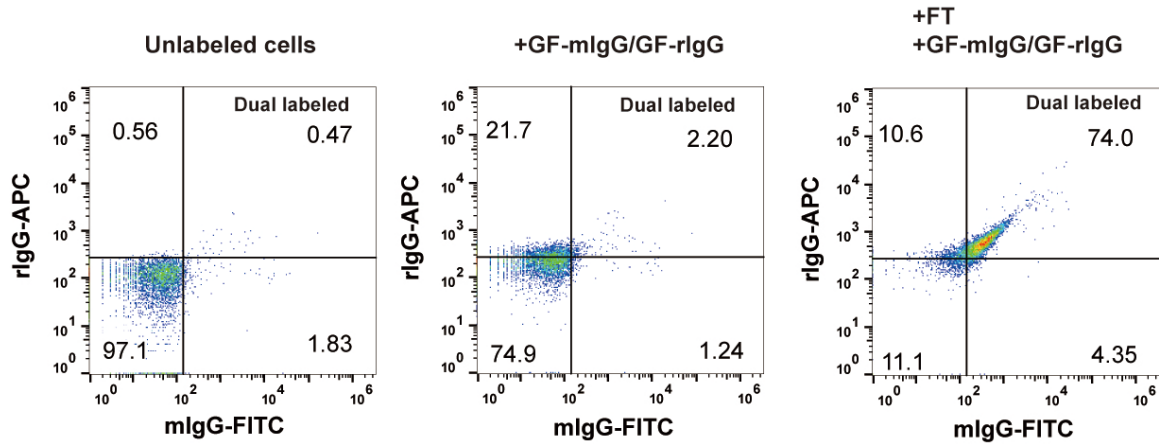


Figure S8. Dual labeling of two different IgG molecules on Lec2 cells. Lec2 cells were treated with GF-mIgG, GF-rIgG and FucT. GF-mIgG and GF-rIgG treated was shown as a negative control. The labeled cells were stained with anti-mIgG-FITC and anti-rIgG-APC antibodies and analyzed by flow cytometry.

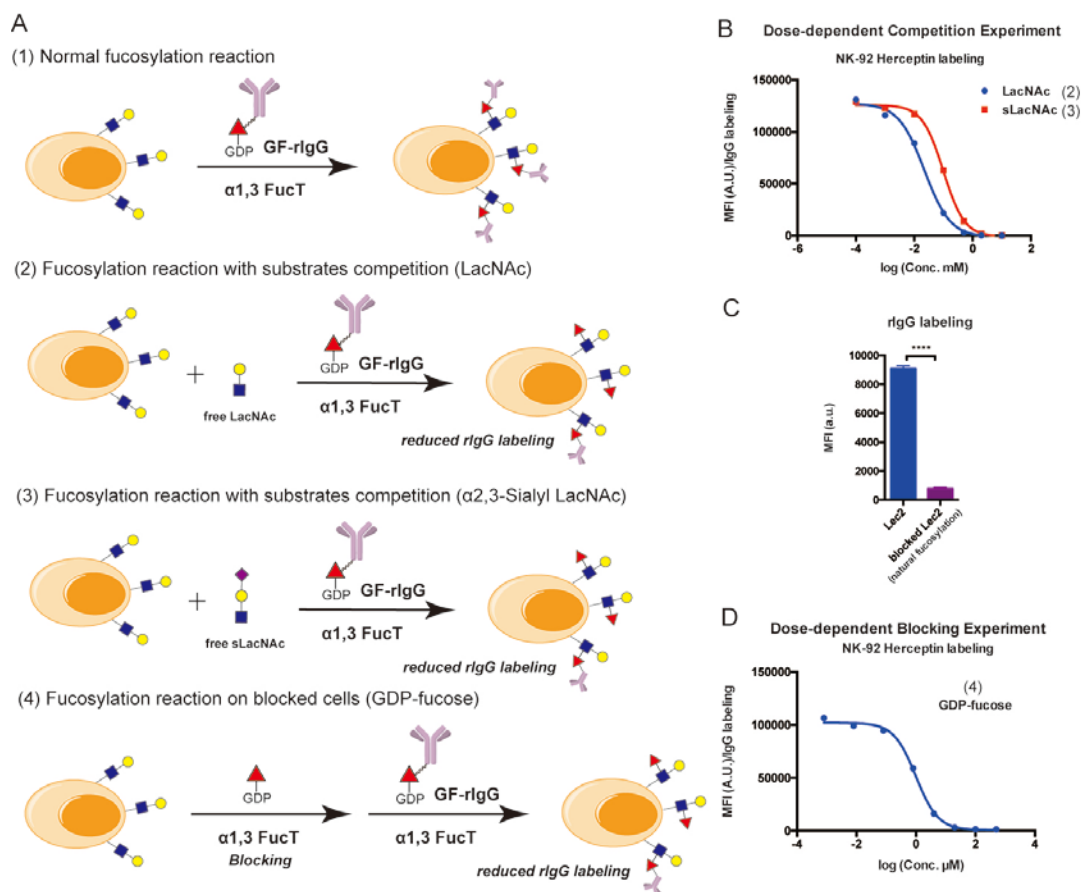


Figure S9. GDP-Fucose or LacNAc analogues inhibit the FucT-mediated transfer of GF-IgG to the cell surface in a dose-dependent manner. (A) Schematic representations of substrate competition assays and reaction sites blocking experiments. (B) Substrates competition experiments, reaction scheme 2 and 3: NK-92MI cells were treated with 60 mU FucT, 0.1 mg/mL GF-IgG and LacNAc or α 2,3 sialyl LacNAc (sLacNAc) with series dilution (varying from 0 to 10 mM) together for half an hour. The fucosylated cells were then stained by a secondary antibody for flow cytometry analysis. (C) Blocking experiments, reaction scheme 4: Lec2 cells were first treated with 30 mU FucT and 100 μ M GDP-Fucose for half an hour. The blocked Lec2 cells were then labeled with 60 mU FucT and 0.1 mg/mL GF-rIgG antibody followed by secondary-antibody staining and flow cytometry analysis. (D) NK-92MI cells were first treated with 30 mU FucT and GDP-Fucose with series dilution (0–500 μ M) for half an hour. The blocked cells were then treated with 60 mU FucT and 0.1 mg/mL GF-IgG, followed by secondary antibody staining and flow cytometry analysis.

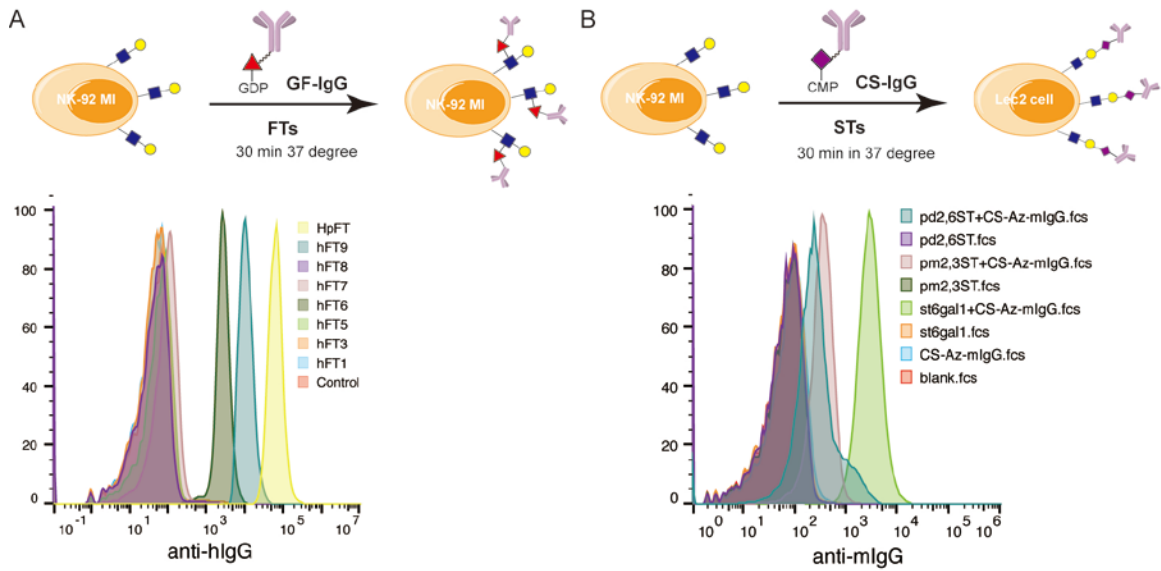


Figure S10. Transfer of IgG to the NK-92MI cell surface enabled by additional fucosyltransferases and sialyltransferases. (A) Comparison of the efficiency of various recombinant human fucosyltransferases with *H. pylori* FucT (HpFT) in transferring GF-IgG to NK-92MI cells. Human FucTs 1,3,5,6,7,8,9 were included. Each enzyme (40 ug/mL) was added with 0.1 mg/mL GF-IgG in the reaction mixture. FucT shows the best efficiency in IgG transfer. Staining antibody: anti-human IgG-APC. (B) Efficiency comparisons between different sialyltransferases in transferring CS-IgG (CMP-Sia conjugated IgG) to NK-92MI cells. Each enzyme (40 ug/mL) was added with 0.1 mg/mL CS-IgG in the reaction mixture. Staining antibody: anti-mouse IgG-APC. Three STs, including recombinant ST6Gal1, *Pasteurella multocida* $\alpha(2,3)$ sialyltransferase M144D mutant (Pm2,3ST-M144D), *Photobacterium damsela* $\alpha(2,6)$ sialyltransferase (Pd2,6ST).

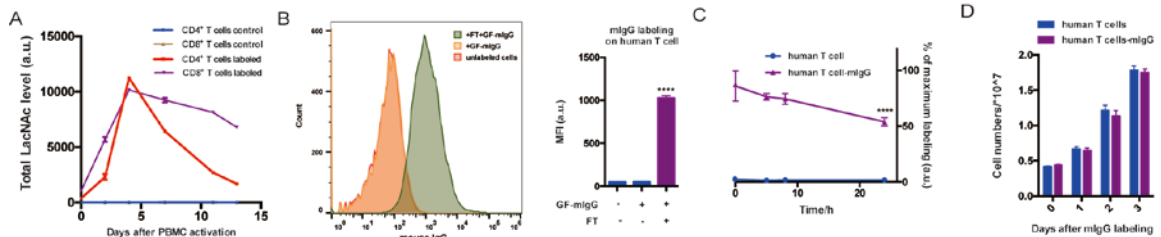


Figure S11. Transferring IgG molecules onto human T cells. (A) LacNAc on Human PBMC was labeled by GF-Biotin on different days after activation. Labeled cells were stained with streptavidin-APC, anti-CD4-PE and anti-CD8-FITC fluorescent antibodies,

and analyzed by flow cytometry. **(B)** Human T cells were treated with FucT and GF-mIgG, or GF-mIgG alone, or untreated. After 15 min of labeling, cells were stained with anti-mIgG-APC antibody and analyzed by flow cytometry. **(C)** Human T cells labeled with mIgG were stained with anti-mIgG-APC antibody and analyzed by flow cytometry at different time points after labeling. **(D)** Human T cells with or without mIgG labeling were cultured in T cell media and live cells in each group were counted on different days after labeling. Error bars, mean values \pm SD. In all figures: ****P < 0.0001; one-way ANOVA followed by Tukey's multiple comparisons test.

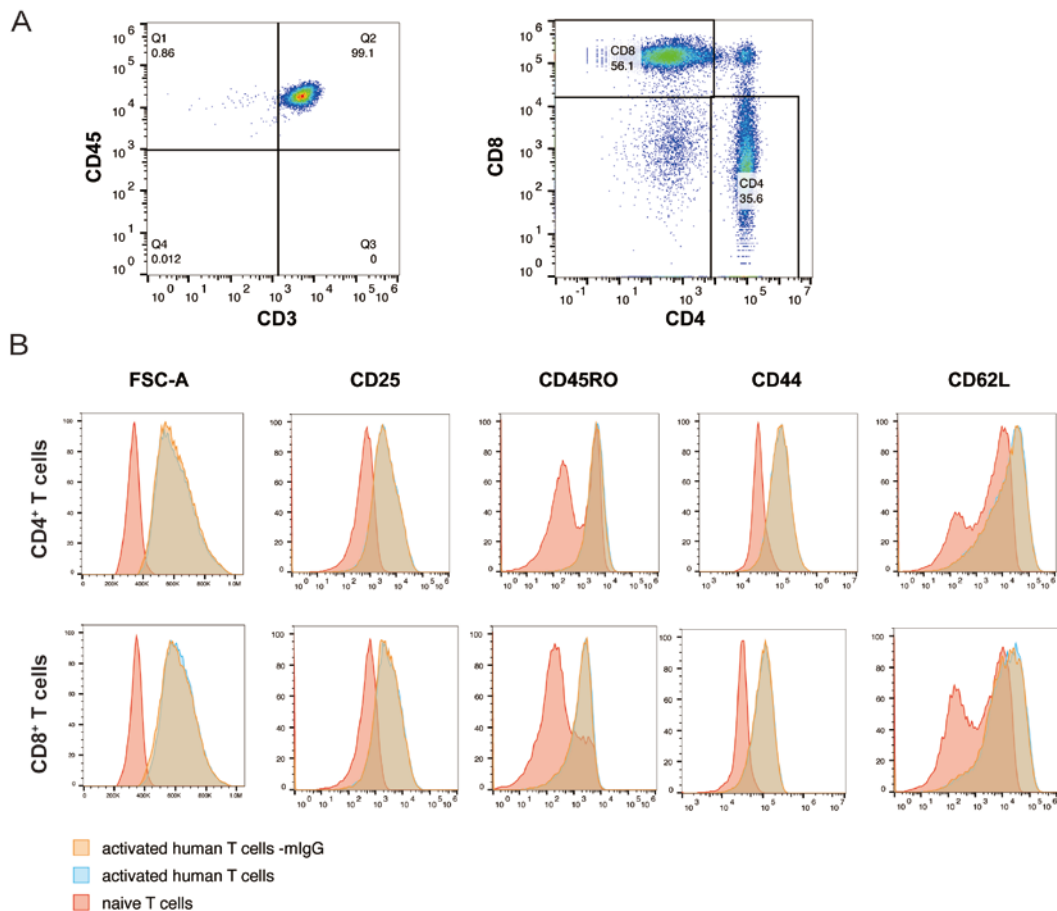


Figure S12. Phenotype of human T cells before and after mIgG labeling. **(A)** Human PBMC were activated by anti-CD3/CD28 antibody and expanded *in vitro* for approximately two weeks. The cells were stained with anti-CD3-APC, anti-CD45-FITC, anti-CD4-PE and anti-CD8-Pacific Blue antibodies, and analyzed by flow cytometry. **(B)** Activated human T cells were treated with GF-IgG and FucT. Labeled or unlabeled cells were stained with anti-CD4-PE/Cy7, anti-CD8-Pacific Blue, anti-CD25-PerCP/Cy5.5,

anti-CD45RO-Alexa Fluor 700, anti-CD44-FITC and anti-CD62L-PE antibodies and analyzed by flow cytometry. Naïve T cells were used as control. CD4⁺ or CD8⁺ human T cells were gated as indicated in (A).

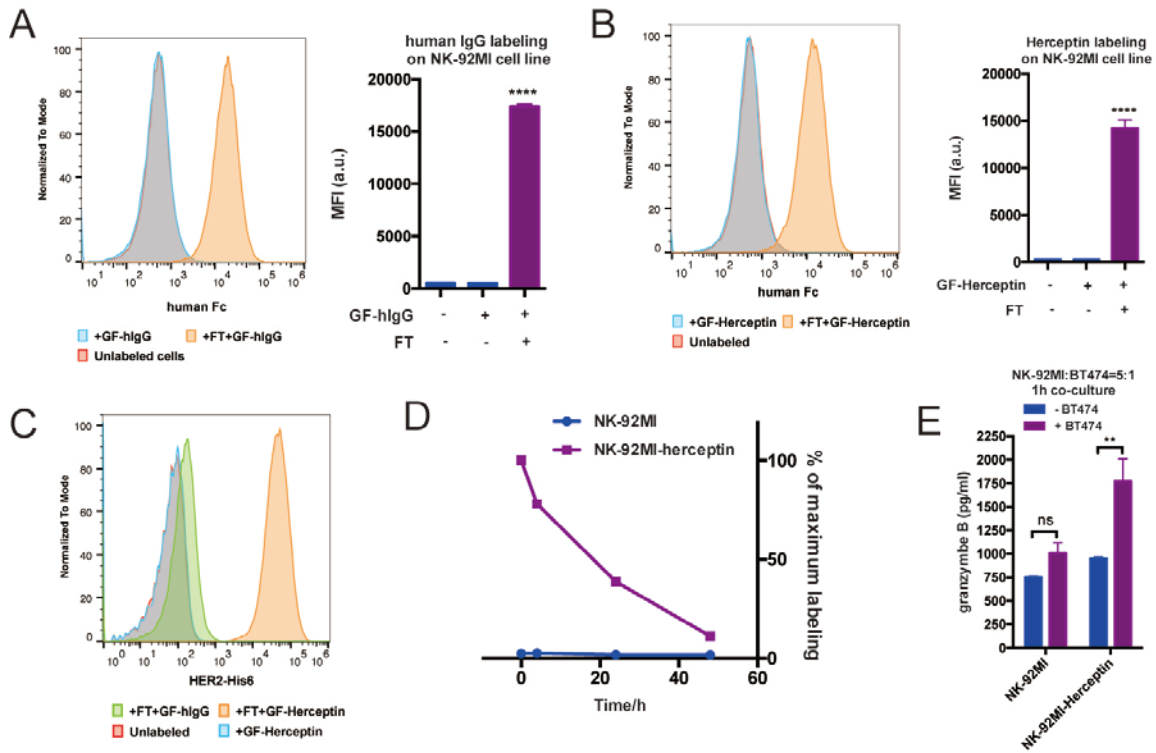


Figure S13. Transferring IgG molecules onto NK-92MI cells. (A) NK-92MI cells were treated with GF-hIgG and FucT, or GF-hIgG alone, or untreated. The cells were stained with anti-hFc APC antibody and analyzed by flow cytometry. (B) NK-92MI cells were treated with GF-Herceptin and FucT, or GF-Herceptin alone, or untreated. The cells were stained with anti-hFc APC antibody and analyzed by flow cytometry. (C) NK-92MI cells with different modifications were then incubated with HER2-His6 protein and then stained with anti-His6-PE antibody for flow cytometry analysis. (D) NK-92MI cells conjugated with Herceptin were stained with anti-hFc APC antibody and analyzed by flow cytometry at different time points post labeling. (E) NK-92MI cells conjugated with or without Herceptin were co-cultured with BT474 at a ratio of 5:1 (Effector : Target) for 1h. Granzyme B secretion in culture supernatant was analyzed by ELISA. Error bars, mean values \pm SD. In all figures: ns, $P > 0.05$; ** $P < 0.01$; one-way ANOVA followed by Tukey's multiple comparisons test (A, B), two-way ANOVA followed by Sidak's multiple

comparisons test (E).

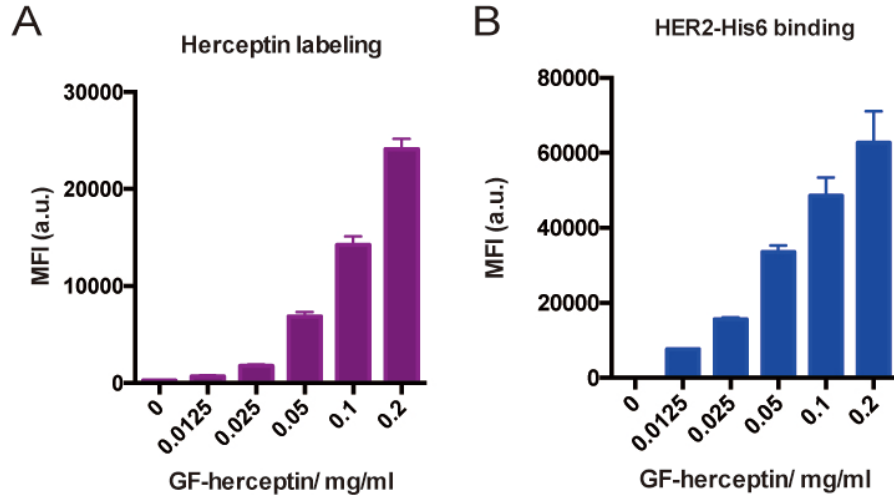


Figure S14. Titration of GF-Herceptin concentrations in the enzymatic transfer. NK-92MI cells were treated with different concentrations of GF-Herceptin in the standard labeling condition. Labeled cells were directly stained with anti-hFc-APC antibody (A), or incubated with HER2-His6 and then stained with anti-His6-PE antibody (B) before flow cytometry analysis.

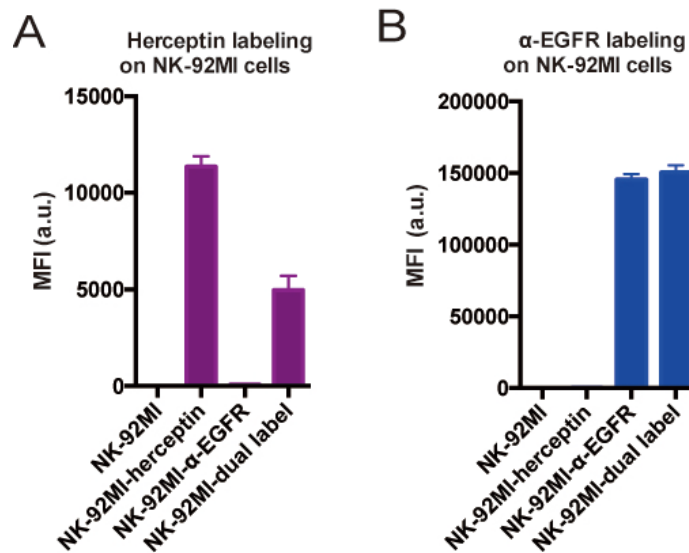


Figure S15. Dual antibody labeling on NK-92MI cells. NK-92MI cells were treated with FucT and GF-Herceptin, and then treated with FucT and GF- α -EGFR. Labeled cells were stained with anti-hFc-APC antibody (A), or stained with anti-mIgG-APC antibody (B) before flow cytometry analysis.

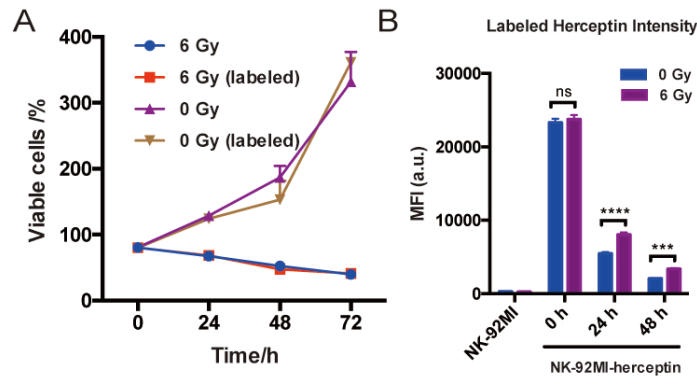


Figure S16. Proliferation and stability of Herceptin-NK-92MI conjugates with or without γ -irradiation. NK-92MI cells were received with or without 6 Gy gamma irradiations before the enzymatic reaction. Labeled and unlabeled NK-92MI cells were cultured for another three days. **(A)** Viable cells were counted by flow cytometry at different time points after irradiation and labeling. **(B)** Herceptin labeled cells were cultured in cell media and then stained with anti-hFc APC antibody and analyzed by flow cytometry at different time points post labeling. Irradiated cells have a slower decay of Herceptin labeling. Error bars, mean values \pm SD. In all figures: ns, $P > 0.05$; *** $P < 0.001$; **** $P < 0.0001$; two-way ANOVA followed by Sidak's multiple comparisons test.

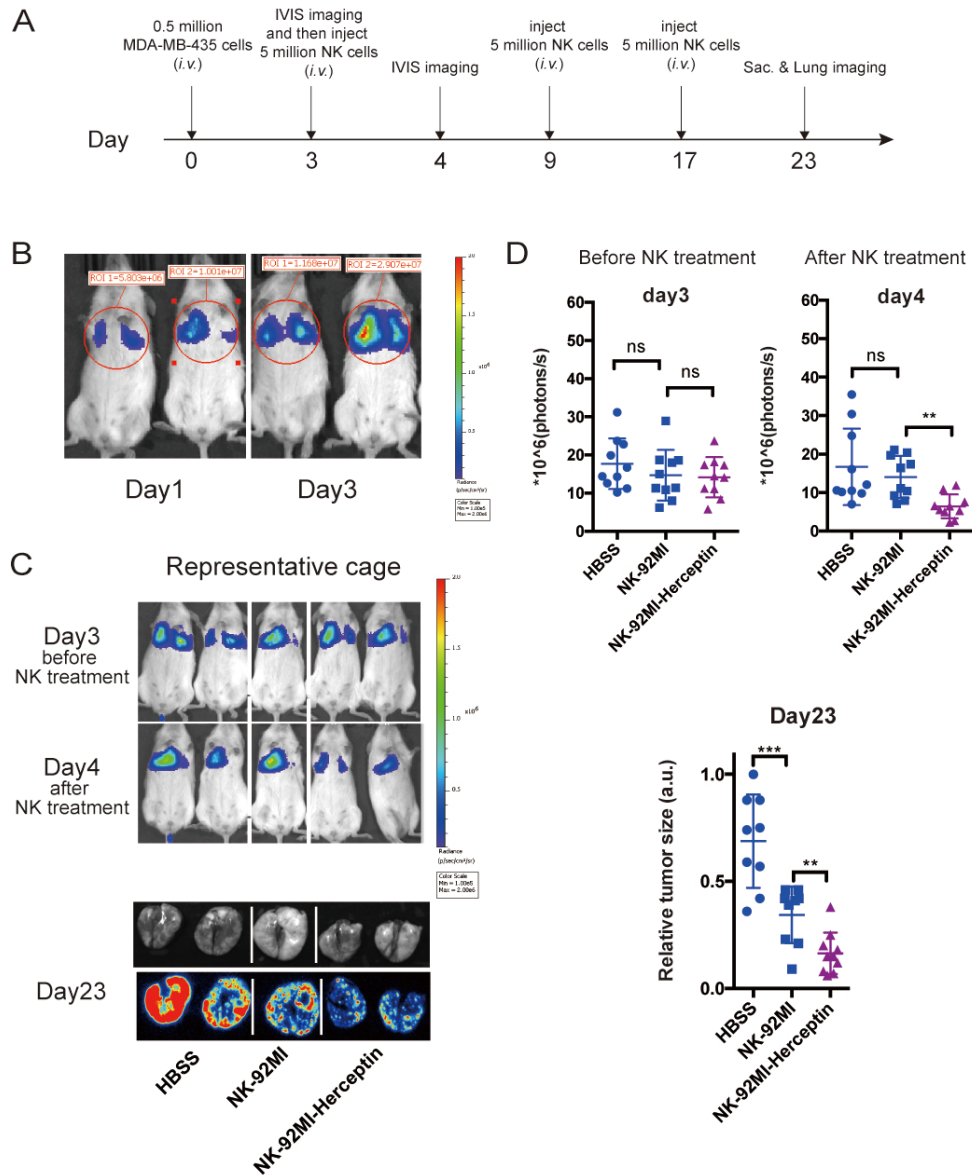


Figure S17. Herceptin-NK-92MI conjugate shows enhanced antitumor activity in established lung metastasis mice model. (A) On day 0, NSG mice were injected intravenously with 0.5 million MDA-MB-435/HER2+/F-luc cells. Three days later, the animals were imaged in IVIS system to detect bioluminescent signals for the confirmation of tumor formation and then treated by *i.v.* injection of 5 million NK-92MI or Herceptin-NK-92MI cells. The control mice received HBSS. The animals were imaged again to see the efficacy of NK cell treatment on day 4. The animals were received one more treatment of 5 million NK-92MI or Herceptin-NK-92MI cells (*i.v.* injection) on day 9 and day 17. The lungs of mice were collected on day 23 and imaged for bioluminescence analysis. (B) Two mice were imaged on day 1 and day3 to show a 3fold

increase in bioluminescence, which confirms that tumors are growing after inoculation. (C) Representative bioluminescent mice images on day 3, day 4 and lung images on day 23 are shown. (D) The sizes of the tumors of the mice and mean values \pm SD are shown; $n = 10$. In all figures, ns, $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$; unpaired t test.

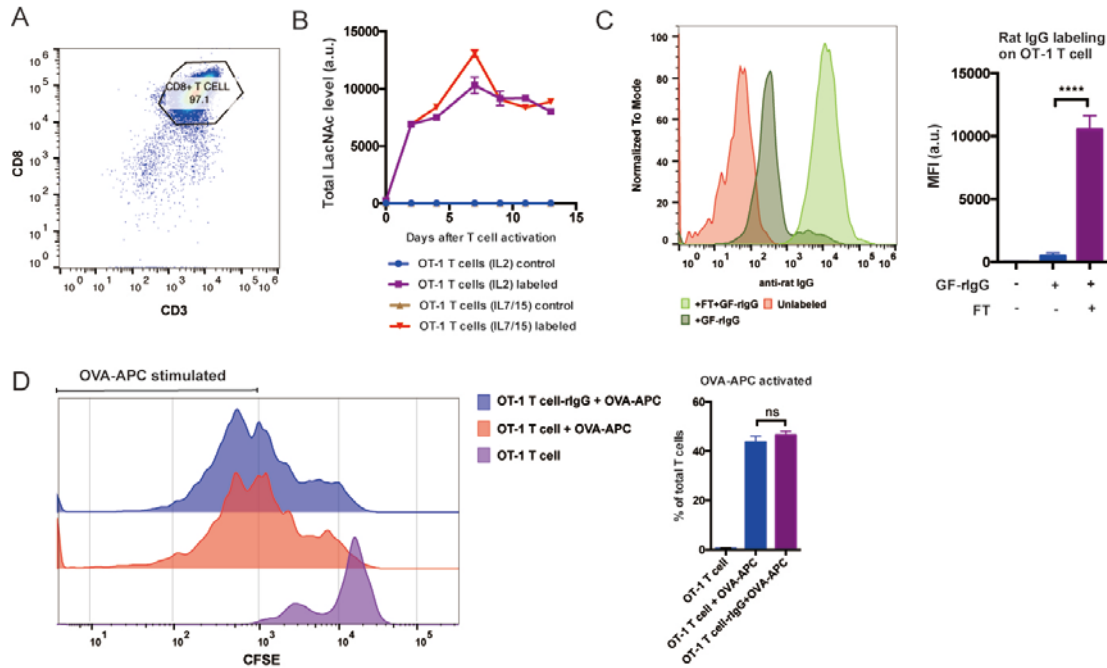


Figure S18. Enzymatic transfer of rIgG to OT-1 CD8⁺ T cells. (A-B) Splenocytes from OT-1 mice were activated by the OVA peptides and *in vitro* expanded by adding IL2 or IL7/IL15. After expansion for four days, the cells were stained with anti-CD3-FITC and anti-CD8-PE antibodies and analyzed by flow cytometry (A). The cells were also treated with GF-A1-Biotin and FucT, and then stained with streptavidin-APC and analyzed by flow cytometry to track the LacNAc level on different days after T cell activation (B). (C) OT-1 T cells were treated with GF-rIgG and FucT, or GF-rIgG alone, or untreated. Then the cells were stained with anti-rIgG-APC antibody and analyzed by flow cytometry. (D) OT-1⁺/-CD45.1⁺/- T cells were first labeled with CFSE and then treated with GF-rIgG and FucT, or untreated. After fucosylation, the cells were co-cultured with OVA peptide pulsed WT B6 splenocytes for 48h and T cell proliferation was analyzed by flow cytometry. Error bars, mean values \pm SD. In all figures: ns, $P > 0.05$; **** $P < 0.0001$; one-way ANOVA followed by Tukey's multiple comparisons test.

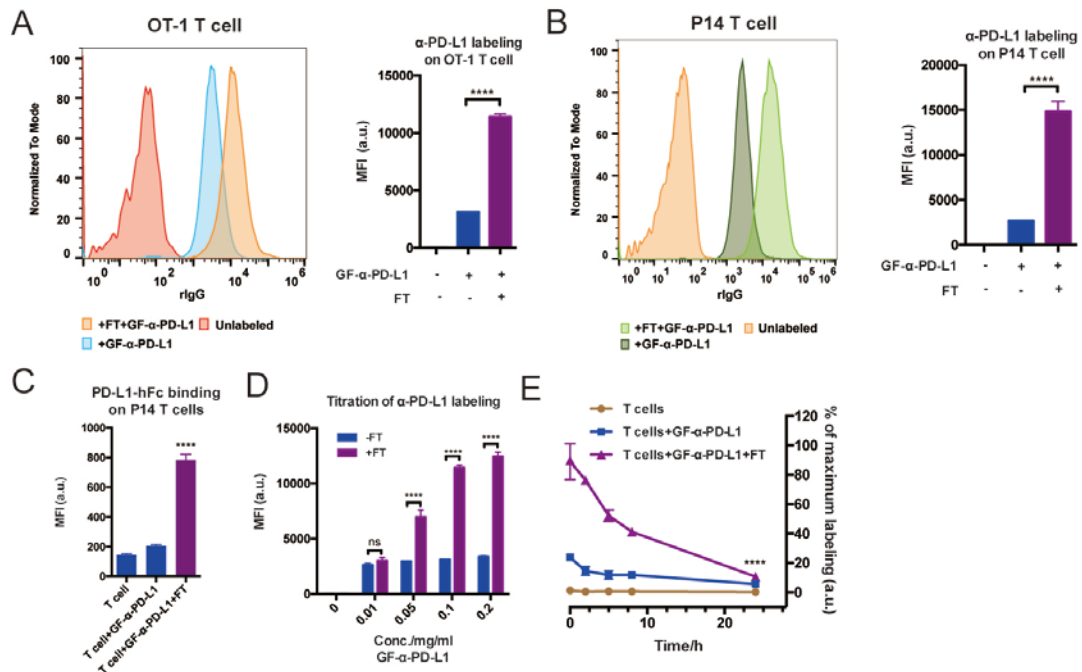


Figure S19. Transferring α -PD-L1 onto OT-1 CD8⁺ T cells. (A) OT-1 CD8⁺ T cells were treated with GF- α -PD-L1 and FucT, or GF- α -PD-L1 alone, or untreated. The cells were then stained with anti-rIgG-APC antibody and analyzed by flow cytometry. (B-C) CD8⁺ T cells from P14 mice were treated with GF- α -PD-L1 and FucT, or GF- α -PD-L1 alone, or untreated. The cells were stained with anti-rIgG APC antibody (B), or incubated with PD-L1-hFc first and then stained with anti-hFc APC antibody (C). After staining, these cells were analyzed by flow cytometry. (D) OT-1 T cells were labeled under different concentrations of GF- α -PD-L1 with or without FucT. After labeling, cells were stained with anti-rIgG-APC antibody and analyzed by flow cytometry. (E) OT-1 T cells were treated with GF- α -PD-L1 and FucT, or GF- α -PD-L1 alone, or untreated. The cells were then stained with anti-rIgG-APC antibody and analyzed by flow cytometry at different time points after reaction. Error bars, mean values \pm SD. In all figures: ns, $P > 0.05$; **** $P < 0.0001$; one-way ANOVA followed by Tukey's multiple comparisons test (A, B, C, E), two-way ANOVA followed by Sidak's multiple comparisons test (D).

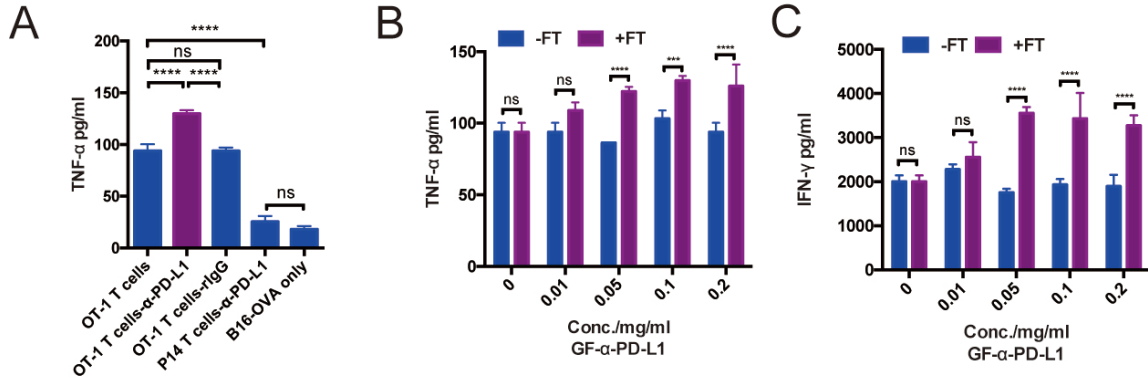


Figure S20. OT-1 T cells modified by α -PD-L1 exhibited enhanced cytokine secretion in the presence of target cancer cells. (A) OT-1 T cells and P14 T cells were treated with GF- α -PD-L1 and FucT, or GF-rIgG and FucT, or untreated. T cells were then co-cultured with B16-OVA cells for 9 hours. TNF- α concentrations in culture supernatant were quantified via ELISA kit. (B-C) OT-1 T cells were treated under different concentrations of GF- α -PD-L1 with or without FucT, and then co-cultured with B16-OVA cells for 9 hours. TNF- α and IFN- γ in culture supernatant were analyzed by ELISA. Error bars, mean values \pm SD. In all figures: ns, $P > 0.05$; *** $P < 0.001$; **** $P < 0.0001$; one-way ANOVA followed by Tukey's multiple comparisons test (A), two-way ANOVA followed by Sidak's multiple comparisons test (B,C).

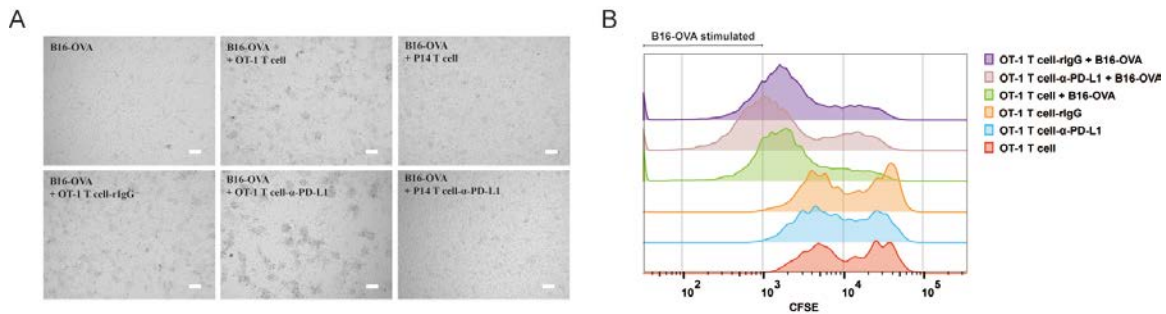
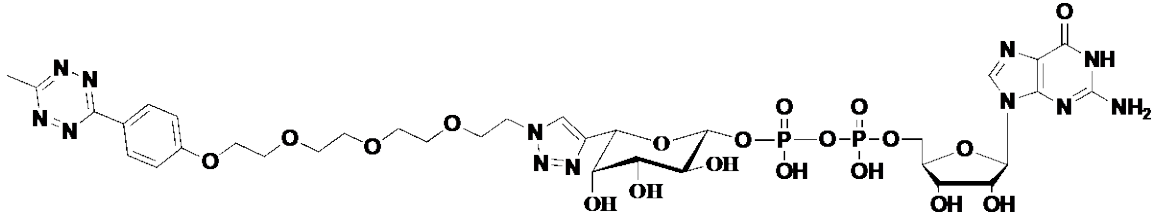


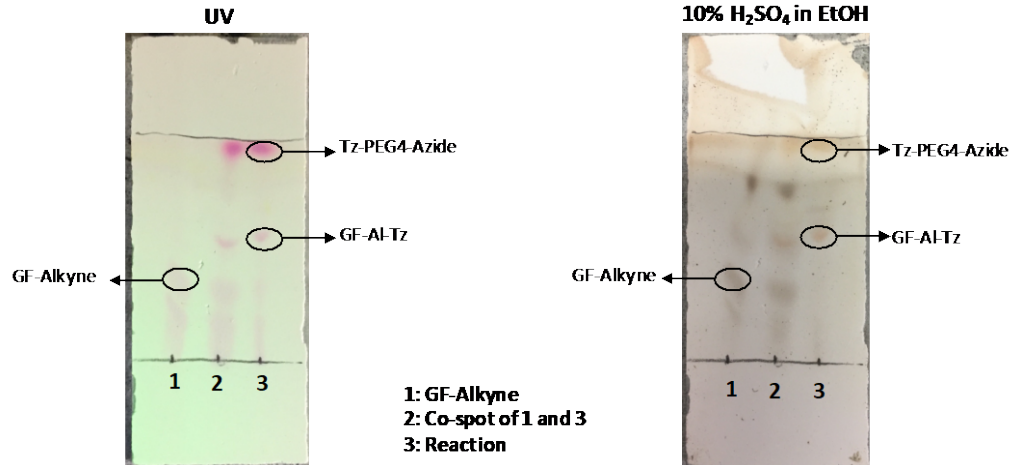
Figure S21. Transferring α -PD-L1 onto T cell surface increases the size of T-cell clustering and T cell proliferation. (A) OT-1 T cells and P14 T cells were treated with GF- α -PD-L1 and FucT, or GF-rIgG and FucT, or untreated. The cells were then co-cultured with B16-OVA cells for 20 hours. T cell cluster during killing were imaged using microscope. Scale bar: 50 μ m. (B) OT-1 T cells were stained with CFSE and treated with GF- α -PD-L1 and FucT, or GF-rIgG and FucT, or untreated. Then the cells were cultured with or without B16-OVA cells for 72 hours. Proliferations of OT-1 T cells were traced by CFSE dilution and flow cytometry analysis.

Spectra

1. Spectra of GF-Al-Tz

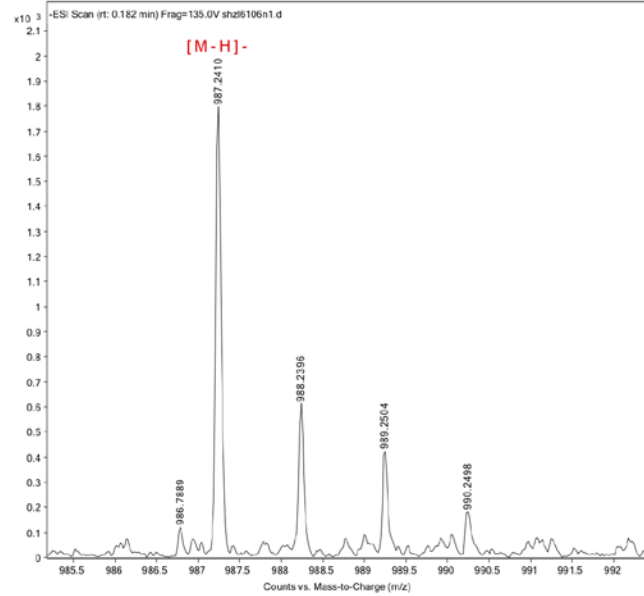


TLC

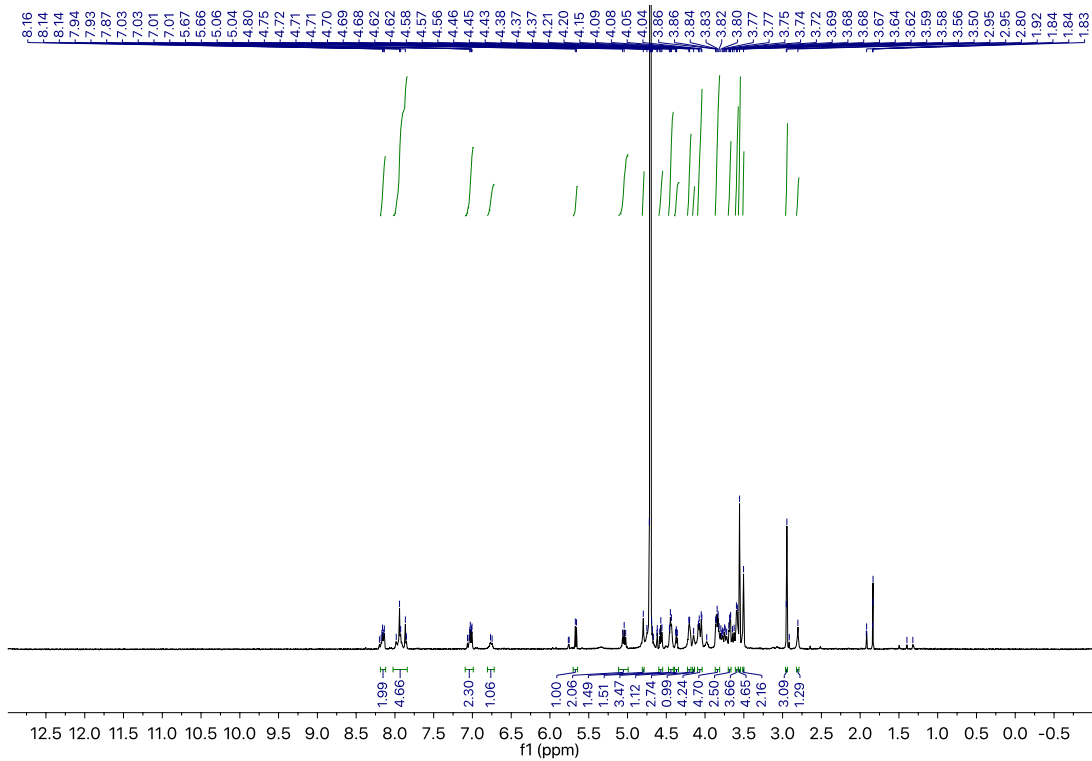


ESI-TOF MS

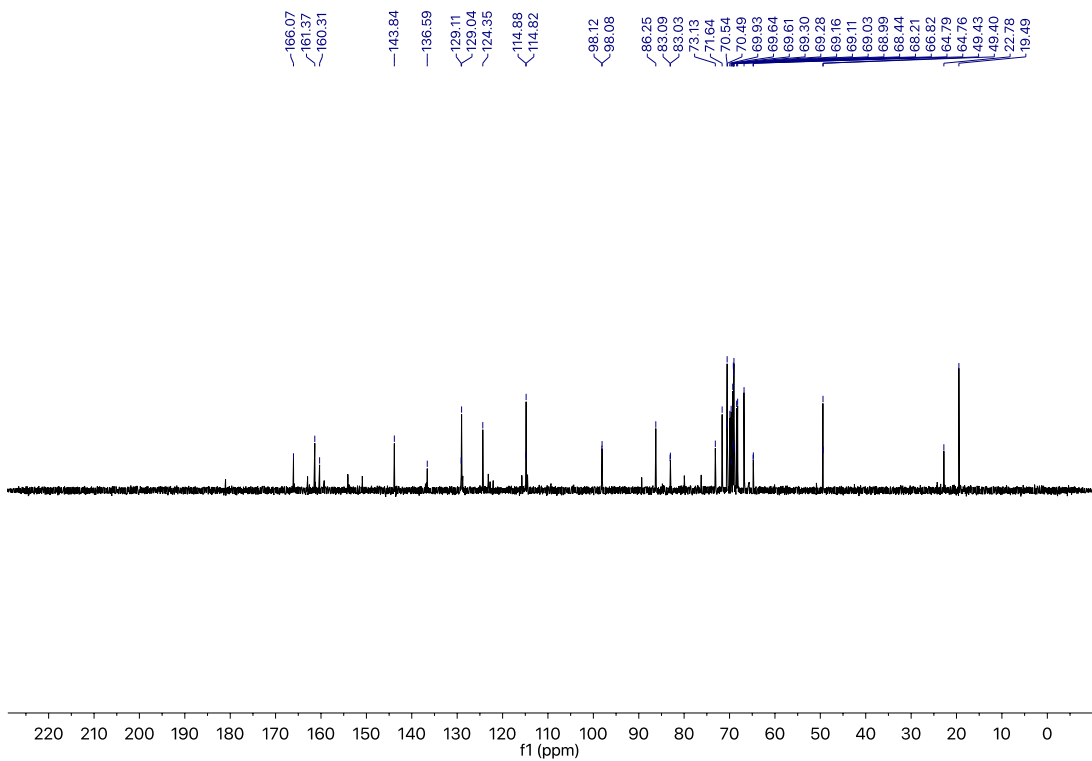
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User Name		Inj Vol	8	Inj Position	
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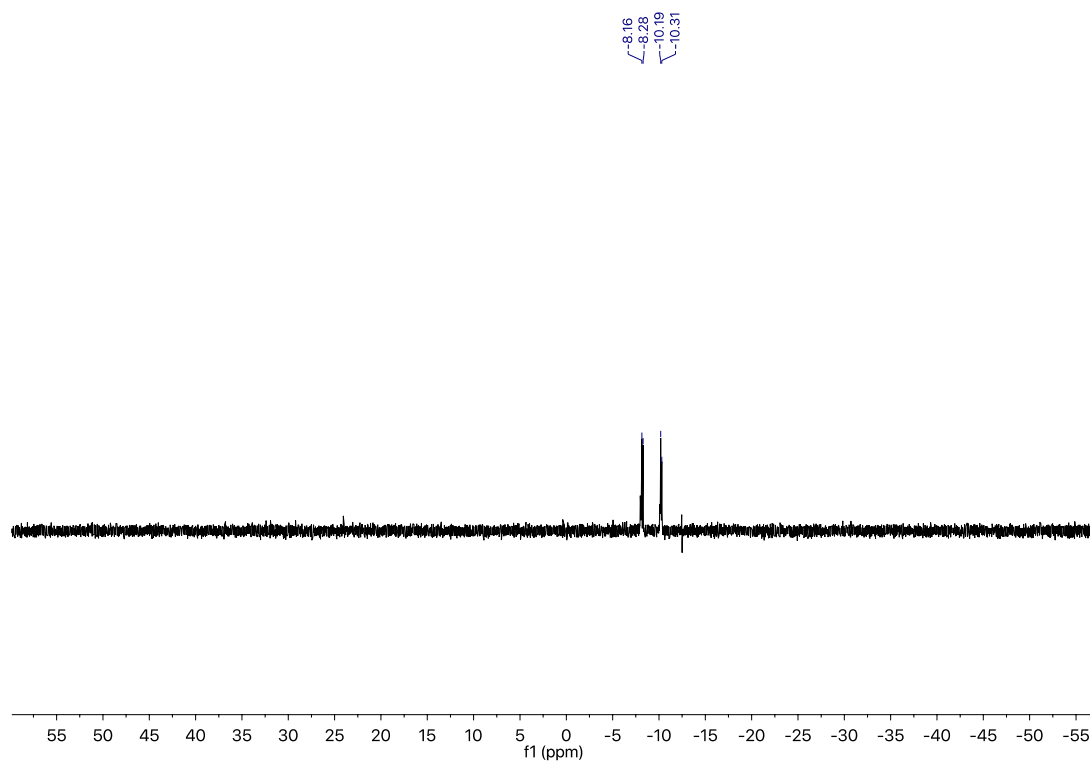
¹H NMR (400 MHz in D₂O)



¹³C NMR (125 MHz in D₂O)

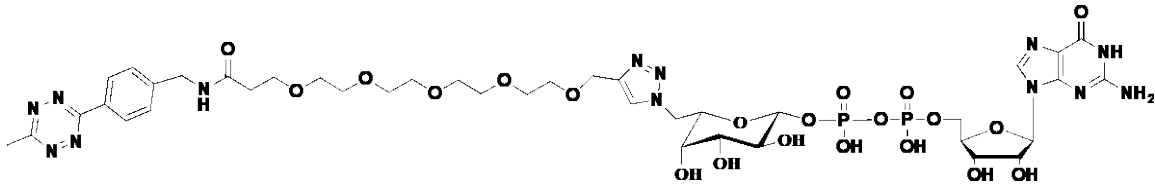


³¹P NMR (161 MHz in D₂O)

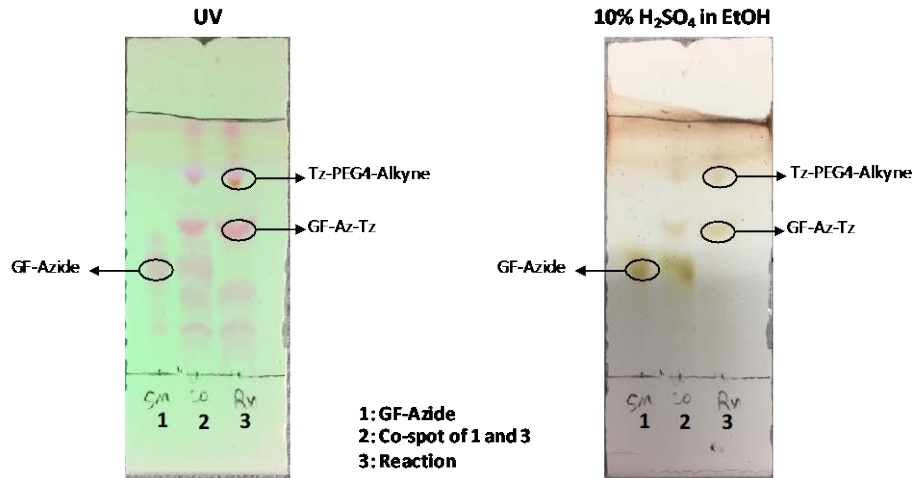


¹H NMR (400 MHz, D₂O): 8.20-8.14 (m, 2H), 7.98-7.85 (m, 4H), 7.06-7.01 (m, 2H), 7.76 (d, 1H, *J* = 12 Hz), 5.67 (d, 1H, *J* = 6 Hz), 5.05 (t, 1H, *J* = 6 Hz), 4.80 (s, 1H), 4.57 (t, 1H, *J* = 6 Hz), 4.47-4.43 (m, 3H), 4.37 (t, 1H, *J* = 6 Hz), 4.46-4.43 (m, 3H), 4.38-4.38 (m, 1H), 4.20 (br m, 2H), 4.15 (br s, 1H), 4.09-4.05 (m, 4H), 3.86-3.82 (m, 4H), 3.69-3.67 (m, 2H), 3.60-3.51 (m, 9H), 2.95 (s, 3H); ¹³C NMR (150 MHz, D₂O): 166.07 (C=O), 161.37, 160.31, 143.84, 136.59, 129.11, 129.04, 124.35, 114.88, 114.82, 98.12, 98.08, 73.13, 71.64, 70.54, 70.49, 69.93, 69.64, 69.61, 69.30, 69.28, 69.16, 69.11, 69.03, 68.99, 68.44, 68.21, 66.82, 64.79, 64.76, 49.43, 22.78, 19.49 19.62 (CH₃); ³¹P NMR (161 MHz, D₂O): -8.22 (d, *J* = 19 Hz), -10.25 (d, *J* = 19 Hz); HRMS (ESI-ve): Found 987.2410 [M - H]⁻; C₃₄H₄₆N₁₂O₁₉P₂ requires 987.2405 [M - H]⁻.

2. Spectra of GF-Az-Tz

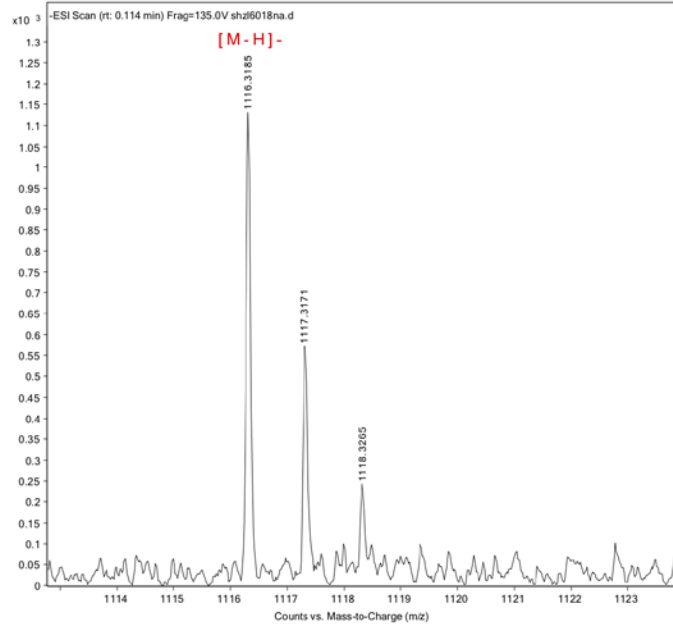


TLC

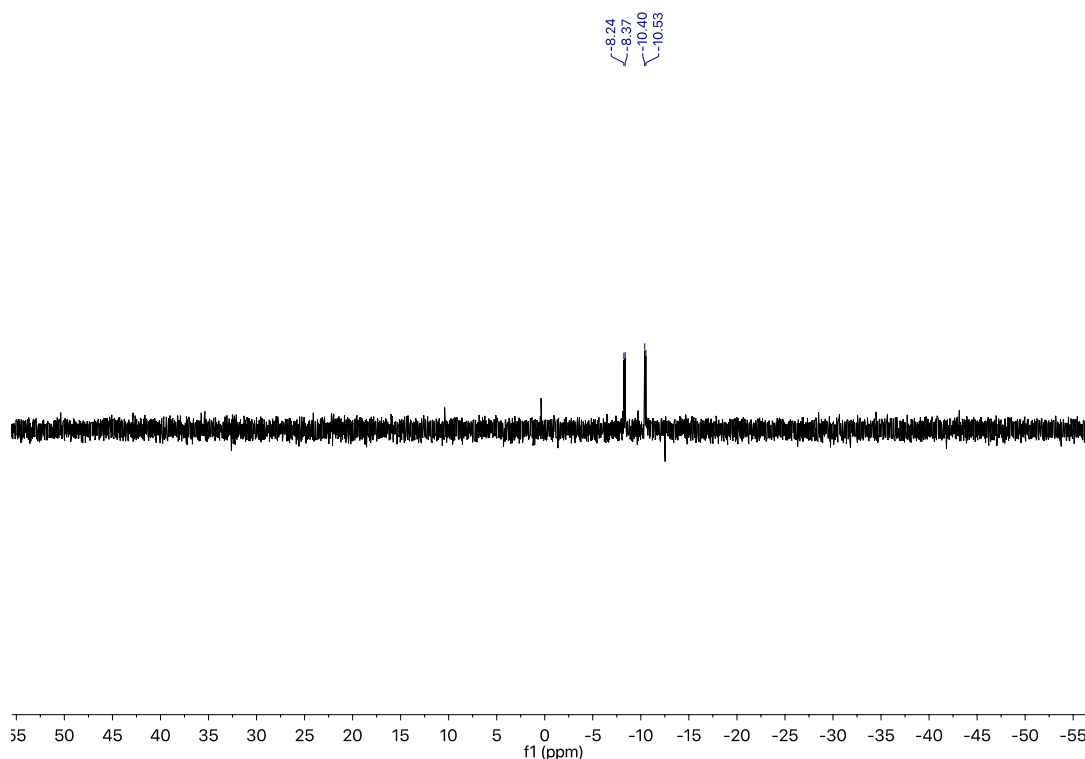


ESI-TOF MS

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ACQ Method	FourIn_20g.m	Comment		Acquired Time	2/16/2018 12:07:34 PM (UTC-08:00)



³¹P NMR (161 MHz in D₂O)



¹H NMR (600 MHz, D₂O): 8.20 (dd, 2H, $J = 6$ Hz, $J = 18$ Hz), 7.98 – 7.89 (m, 4H), 7.44 – 7.37 (m, 2H), 7.27 (d, 1H, $J = 6$ Hz), 5.73 (dd, 2H, $J = 6$ Hz, $J = 24$ Hz), 4.83 – 4.78 (m, 1H), 4.63 – 4.44 (m, 7H), 4.33 – 4.10 (m, 6H), 3.92 – 3.87 (m, 2H), 3.76 – 3.68 (m, 3H), 3.61 – 3.46 (m, 9H), 2.97 (s, 2H), 2.87 (br s, 1H), 2.64 (s, 2H), 2.56 – 2.52 (m, 2H); ¹³C NMR (150 MHz, D₂O): 173.85 (C=O), 166.70 (C=O), 163.21, 153.33, 142.59, 129.42, 127.44, 127.39, 125.33, 98.57, 97.93, 88.48, 86.21, 83.08, 83.02, 73.10, 72.95, 71.57, 71.54, 70.51, 70.46, 69.85, 69.12, 69.11, 69.06, 69.05, 69.03 (2 x C), 69.00, 68.39, 68.37, 68.00, 66.34, 62.44, 62.00, 49.67, 42.27, 38.24, 35.67, 19.62 (CH₃); ³¹P NMR (161 MHz, D₂O): -8.31 (d, $J = 21$ Hz), -10.47 (d, $J = 21$ Hz); HRMS (ESI-ve): Found 1116.3194 [M – H]⁻; C₄₀H₅₇N₁₃O₂₁P₂ requires 1116.3185 [M – H]⁻.

Reference

1. Wang, W., Hu, T., Frantom, P.A., Zheng, T., Gerwe, B., del Amo, D.S., Garret, S., Seidel, R.D. & Wu, P. Chemoenzymatic Synthesis of GDP-l-fucose and the Lewis X Glycan Derivatives. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16096-16101.
2. Besanceney-Webler, C., Jiang, H., Zheng, T., Feng, L., Soriano del Amo, D., Wang, W., Klivansky, L.M., Marlow, F.L., Liu, Y. & Wu, P. Increasing the Efficacy of Bioorthogonal Click Reactions for Bioconjugation: a Comparative Study. *Angew. Chem. Int. Ed.* **2011**, *50*, 8051-8056.
3. Chen, L., Cohen, J., Song, X., Zhao, A., Ye, Z., Feulner, C.J., Doonan, P., Somers, W., Lin, L. & Chen, P.R. Improved Variants of SrtA for Site-specific Conjugation on Antibodies and Proteins with High Efficiency. *Scientific Reports* **2016**, *6*, 31899.

4. Jeong, H.-J., Abhiraman, G.C., Story, C.M., Ingram, J.R. & Dougan, S.K. Generation of Ca²⁺-Independent Sortase A Mutants with Enhanced Activity for Protein and Cell Surface Labeling. *PLOS ONE* **2017**, *12*, e0189068.
5. Rahim, M.K., Kota, R. & Haun, J.B. Enhancing Reactivity for Bioorthogonal Pretargeting by Unmasking Antibody-Conjugated trans-Cyclooctenes. *Bioconjug. Chem.* **2015**, *26*, 352-360.
6. Rondon, A., Ty, N., Bequignat, J.-B., Quintana, M., Briat, A., Witkowski, T., Bouchon, B., Boucheix, C., Miot-Noirault, E., Pouget, J.-P., Chezal, J.-M., Navarro-Teulon, I., Moreau, E. & Degoul, F. Antibody PEGylation in Bioorthogonal Pretargeting with Trans-cyclooctene/Tetrazine Cycloaddition: In Vitro and In Vivo Evaluation in Colorectal Cancer Models. *Scientific Reports* **2017**, *7*, 14918.