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Immunoengineering can overcome the glycocalyx armour of cancer cells

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SUPPLEMENTARY NOTE

Supplementary Note 1: Scanning angle interference microscopy

Scanning Angle Interference Microscopy is a fluorescence localization microscopy technique with nanometer scale axial precision. In SAIM, a reflective silicon mirror is used as the sample substrate and fluorescence is excited by laser illumination at precisely controlled incidences angles. Reflection from the mirrored substrate creates a periodic interference pattern along the optical axis by superposition of the direct and reflected excitation light, the spatial frequency of which depends on the angle of incidence of the light on the sample. Because the fluorescence emission intensity is proportional to the excitation light intensity, the regularly spaced series of maxima and minima in the interference pattern projected on the sample lead to an observable height-dependent variability in fluorescence intensity from the sample. Capturing a series of images at known incidence angles allows precise localization based upon variations in observed intensity in a given pixel. By fitting the measured emission intensities in the image series to an optical model, SAIM enables the precise localization of fluorescent reporters with nanoscale axial precision. To improve the precision of SAIM, in this work, we use a pair of high-speed, galvanometer-controlled mirrors to continuously scan the excitation laser azimuthally about the optical axis while maintaining the sample incidence angle[6]. This approach, which we refer to as Ring-SAIM, is intended to improve sample illumination and, thus, the precision of measurement compared to standard SAIM implementations[6].

Sample preparation

Silicon wafers with ~1900 nm thermal oxide (Addison Engineering) were diced into 7 mm by 7 mm chips, and the oxide layer thickness for each chip was measured with a FilMetrics F50-EXR.

Prior to use, the chips were then cleaned with 1:1 methanol and hydrochloric acid for 15 minutes, followed by 1 minute in a plasma cleaner (Harrick Plasma, PDC-001). The chips were incubated with 4% (v/v) (3-mercaptopropyl)trimethoxysilane in absolute ethanol for 30 minutes at room temperature. After washing with absolute ethanol, the chips were subsequently incubated with 4 mM 4-maleimidobutyric acid N-hydroxysuccinimide ester in absolute ethanol for 30 minutes and rinsed with PBS. 50 μg/ml fluorescent labelled human plasma fibronectin in PBS was incubated with the functionalized chips overnight at 4°C for conjugation. Cells were seeded onto the chips at 2×10^4 cells/cm^2 in growth medium with varying doxycycline concentrations and incubated for 24 hours. Cells expressing different lengths of Muc1 were labelled with GFP nanobody conjugated to Alexa Fluor 647 in MCF10A growth media for 10 minutes at 37°C. 1E7 and B2 cells were labelled with MemGlow dye (MemGlow 560: MG02-2; MemGlow 590: MG03-10; MemGlow 640: MG04-02, Cytoskeleton) or CellBrite Steady 550 (#30107-T, biotium) in serum-free, phenol red-free DMEM/F12 for 10 minutes at 37°C. Samples were then rinsed three times in PBS, inverted into a 35 mm glass-bottom imaging dish containing imaging buffer (MemGlow dye or CellBrite dye: serum-free, phenol red-free DMEM/F12, Alexa Fluor 647 conjugated GFP nanobody: phenol red-free MCF10A growth media) and imaged at 37°C.

Optical setup

Scanning angle interference microscopy (SAIM) was conducted on a custom circle-scanning microscope.[6] The core of the setup was an inverted fluorescence microscope (Ti-E, Nikon). The excitation lasers (488 nm, Coherent; 560 nm, MPB Communications Inc.; and 642 nm, MPB Communications Inc.) were combined into a colinear beam by a series of dichroic mirrors (Chroma). The combined output beam was attenuated and shuttered by an AOTF (AA Opto-Electronic). The beam was directed onto a pair of galvanometer scanning mirrors (Cambridge

Technology). The image of the laser on the scanning mirrors was magnified and relayed to the sample by two 4F lens systems, a beam expanding telescope and a scan lens / objective lens combination. The beam expander was formed by $f = 30$ mm and $f = 300$ mm achromatic lenses with a m = 1 zero-order vortex half-wave plate positioned between them and positioned $2f$ from the 300 mm achromatic scan lens (Thorlabs). SAIM experiments were performed with a 60x N.A. 1.27 water immersion objective (Nikon). Fluorescence emission was collected with a quad band filter cube and single band filters (TRF89901-EMv2, Chroma) mounted in a motorized filter wheel (Sutter). Images were acquired with a Zyla 4.2 sCMOS (Andor) camera or an iXon 888 EMCCD (Andor) using the microscope's 1.5x magnifier for a total magnification of 90×. The open-source software Micro-Manager was used for camera and filter wheel control and image acquisition[7]. The circle-scanning galvometers were operated in an autonomous fashion using a custom-designed 16-bit PIC microcontroller, which has been described previously[6].

Image Acquisition and Analysis

For Ring-SAIM, 32 images were acquired at varying incidence angles for the circle-scanned excitation beam. During a typical image acquisition sequence, changes in the scanned incidence angle were triggered by a TTL signal from the camera to the microcontroller. The incidence angles were evenly spaced from 5 to 43.75 degrees with respect to the wafer normal in the imaging media. To obtain the reconstructed height topography of the samples, the raw image intensities, I_j, at each incidence angle, θ_j, were fit pixelwise by nonlinear least-squares to an optical model:

$$I_j = A * f(\theta_j, H) + B \tag{1}$$

where H is the unknown sample height and A and B are additional fit parameters. The vortex half-wave plate in the optical setup maintained the s-polarization of the circle-scanned excitation laser. For s-polarized monochromatic excitation of wavelength, λ, the probability of excitation, $f(\theta_j, H)$, for the system is given by:

$$f(\theta_j, H) = 1 + 2Re\{r^{TE}\}\cos\phi - 2Im\{r^{TE}\}\sin\phi + Re\{r^{TE}\}^2 + Im\{r^{TE}\}^2 \tag{2}$$

where the phase shift, ϕ, and the reflection coefficient for the transverse electric wave, r^{TE}, are given by:

$$\phi(H) = \frac{4\pi}{\lambda}(n_b H \cos\theta_b) \tag{3}$$

$$r^{TE} = \frac{(m_{11}^{TE} + m_{12}^{TE} p_{Si})p_2 - (m_{21}^{TE} + m_{22}^{TE} p_0)}{(m_{11}^{TE} + m_{12}^{TE} p_{Si})p_2 + (m_{21}^{TE} + m_{22}^{TE} p_0)} \tag{4}$$

$$M^{TE} = \begin{pmatrix} m_{11}^{TE} & m_{12}^{TE} \\ m_{21}^{TE} & m_{22}^{TE} \end{pmatrix} = \begin{pmatrix} \cos(k_{ox} d_{ox} cos\theta_{ox}) & -\frac{i}{p_1}\sin(k_{ox} d_{ox} cos\theta_{ox}) \\ -ip_1 \sin(k_{ox} d_{ox} cos\theta_{ox}) & \cos(k_{ox} d_{ox} cos\theta_{ox}) \end{pmatrix} \tag{5}$$

$$p_0 = n_{Si} cos\theta_{Si}, p_1 = n_{ox} cos\theta_{ox}, p_2 = n_b cos\theta_b \tag{6}$$

$$k_i = \frac{2\pi n_i}{\lambda}, \theta_{ox} = sin^{-1}\frac{n sin\theta_b}{n_{ox}}, \theta_{Si} = sin^{-1}\frac{n sin\theta_{ox}}{n_{Si}} \tag{7}$$

where k_i is the wavenumber in material i; n_{Si}, n_{ox} and n_b are the refractive index of the silicon, silicon oxide and sample, respectively; θ_{Si}, θ_{ox} and θ_b are the angles of incidence in the silicon, silicon oxide and sample, respectively; and d_{ox} is the thickness of the silicon oxide layer. The angles of incidence in silicon oxide and silicon were calculated according to Snell's Law. To quantify the glycocalyx thickness of cells, the average height above the silicon substrate was calculated for a 100 x 100 pixel subregion in each cell. The glycocalyx thickness was reported as the height of the localized anti-GFP nanobody, MemGlow dye or CellBrite dye signal minus the height of the fluorescently labelled fibronectin layer on the silicon substrate.

Supplementary Note 2: Preparation of recombinant human galectins

Human *LGALS1* and *LGALS3* constructs in pET21 were recombinantly expressed in *E. coli* strain NiCo21 (DE3) (New England Biolabs). Transformed bacteria were grown in LB at 37°C until an OD600 of 0.6 – 0.8 was reached. Expression was induced with 0.3 mM IPTG, and protein was produced overnight at 20°C. Cells were harvested by centrifugation at 3,000 g for 20 minutes and lysed by a pressurized homogenizer with cOmplete protease inhibitor Cocktail (Roche) and 1 mg/ml lysozyme (Sigma). The lysate was centrifuged at 10,000 g for 45 minutes at 4°C, and the supernatant was incubated with β-lactosyl Sepharose resin for 1 hour at 4°C before loading into a gravity column. The protein was eluted with 0.1 M β-lactose (Santa Cruz Biotech) and 8 mM DTT (Sigma). The partially purified protein was polished on a HiPrep 16/60 Sephacryl S-100 HR (GE Healthcare Life Sciences) column equilibrated with 0.1 M β-lactose and 8 mM DTT. The final protein was then concentrated by using Amicon Ultra (3kD MWCO for Galectin-1; 10kD MWCO for Galectin-3) filters (Millipore Sigma). Conjugation of β-lactose to Sepharose 6B (Sigma) to synthesize the β-lactosyl Sepharose was as previously described[1]. For fluorescent galectins, purified galectins were labeled with Alexa Fluor 568 NHS Ester (Thermo Fisher Scientific) per the manufacturer's protocol.

Supplementary Note 3: Preparation and conjugation of recombinant GFP nanobody

For recombinant GFP nanobody, cDNA (PDB #3OGO_E) was generated through custom gene synthesis and inserted in the pTP1112 plasmid (Addgene #104158). Nanobody was recombinantly produced in chemically competent NiCo21 (DE3) E. coli (NEB) grown in LB at 37°C until an OD600 of 0.6 was reached, at which time the cultures were induced with 0.5 mM IPTG and grown overnight at 24°C. Harvested cells were lysed in B-PER (Thermo Fisher Scientific), and the lysates were cleared by centrifugation at 10,000 g for 20 minutes at 4°C. The His-tagged nanobody was purified by IMAC according to standard protocols. Briefly, supernatant diluted into 1x Ni-NTA binding buffer was bound to equilibrated Ni-NTA resin (Qiagen, 30210) for 20 minutes at 4°C, with end-over-end mixing. The resin was added to a spin column, washed thoroughly, and incubated with the Ni-NTA elution buffer for 20 minutes at 4°C, mixing end-over-end. Eluted protein was exchanged into storage buffer (pH 7.4 PBS) using Zeba 7K MWCO desalting columns or by overnight dialysis with 10K MWCO Snakeskin dialysis tubing. Eluted proteins were then sterile filtered and snap-frozen for long-term storage at -80°C. Nanobody-containing constructs were mixed with 0.1% w/v sodium azide prior to snap-freezing. For fluorescent GFP nanobody, purified GFP nanobody was labeled with Alexa Fluor 647 NHS Ester (Thermo Fisher Scientific) per the manufacturer’s protocol.

Supplementary Note 4: Preparation of recombinant mucinases and sialidase

The cDNA for StcE-Δ35[2] was synthesized by custom gene synthesis (Twist Bioscience) and inserted into the pET28b expression vector (See Supplemental Table 4). StcE E447D was generated through mutation of StcE-Δ35 using Q5 Site-Directed Mutagenesis Kit (New England Biolabs) with primers 5'-TCAGTCATGACGTTGGTCATAATTATG-3' and 5'-ACTCATTCCCCAATGTGG-3'. StcE-ΔX409 was generated through mutation of StcE-Δ35 using the Q5 Site-Directed Mutagenesis Kit with primers 5'- TAACTCGAGCACCACCAC-3' and 5'- ATTTACAGTATAGGTAAGTCCTTC-3' (See Supplemental Table 4). The cDNA for *Salmonella typhimurium* sialidase and Sialidase-EE was synthesized by custom gene synthesis (Twist Bioscience) and inserted into the pET28b expression vector (See Supplemental Table 4). The general design of StcE-EE is as follows[3,4]. The 35-aa glycine/serine $(GGGGS)_7$ linker and leucine zipper (EE zip) construct was synthesized by custom gene synthesis (Twist Bioscience) and inserted into the pET28b StcE ΔX409 vector using NEBbuilder HiFi DNA Assembly (New England Biolabs) with primers 5'- TGAGATCCGGCTGCTAAC-3' and 5'-ATTTACAGTATAGGTAAGTCCTTCTG-3'. Recombinant protein was produced in NiCo21 (DE3) as described for recombinant GFP nanobody above. Cells were harvested by centrifugation at 3,000 g for 20 minutes, resuspended in lysis buffer (20 mM HEPES, 500 mM NaCl and 10 mM imidazole, pH 7.5) with cOmplete protease inhibitor Cocktail (Roche), and lysed by a pressurized homogenizer. Mucinase was purified by immobilized metal affinity chromatography (IMAC) on a GE ÄKTA Avant FPLC system. The lysate was loaded onto a HisTrap HP column (GE Healthcare Life Sciences), washed with 20 column volumes of wash buffer (20 mM HEPES, 500 mM NaCl and 20 mM imidazole, pH 7.5.), and eluted with a linear gradient of 20 mM to 250 mM imidazole in buffer (20 mM HEPES and 500 mM NaCl, pH 7.5.).

The elution fractions containing target protein were collected and polished on a HiPrep 26/60 Sephacryl S-200 HR (GE Healthcare Life Sciences) column equilibrated with storage buffer (20 mM HEPES and 150 mM NaCl, pH 7.5). The final protein was then concentrated by using Amicon Ultra 30 kDa MWCO filters (Millipore Sigma)[5].

SUPPLEMENTARY TABLE

Supplementary Table 1. Antibody and Lectin reagent information table

Reagent	Source	Identifier (Cat#)
Antibody or Lectin		
Anti-Human Muc1 Clone HMPV	BD Biosciences	555925
Anti-Human Muc1 Janelia Fluor 549	Novus Biologicals	NBP-2-47883JF549
Recombinant anti-GNE antibody	Abcam	ab189927
Anti-GCNT1 antibody	Abcam	ab102665
Anti-Galectin-3	Santa Cruz Biotechnology	sc-19280
Mouse anti-β-Actin Clone C4	Santa Cruz Biotechnology	sc-47778
Anti-ErbB2/HER2 Clone 3B5	Abcam	ab16901
APC conjugated anti-Human perforin	BioLegend	30811
Anti-6X His-tag antibody	Abcam	ab9108
Anti-CD19 antibody	Abcam	ab134114
APC conjugated anti-human CD328	BioLegend	339205
Anti-human Siglec-9	R&D systems	MAB1139-SP
PE-Vio770 conjugated anti-human CD56	Miltenyi Biotec	130-113-870
FITC conjugated anti-human CD3	Miltenyi Biotec	130-113-690
Goat anti-mouse IgG DyLight 800 4x PEG conjugate	Invitrogen	SA535521
Goat anti-mouse IgG DyLight 680 conjugate	Invitrogen	35518
Goat anti-rabbit IgG Alexa Fluor 647 conjugate	Thermo Fisher Scientific	A21245
Goat anti-mouse Alexa Fluor 647 conjugate	Invitrogen	A28181
Rabbit anti-goat IgG Alexa Fluor 647 conjugate	Invitrogen	A21446
CF640R-conjugated PNA	Biotium	29063
Biotin-conjugated MAL-II	Vector Laboratories	B-1265-1
Biotin-conjugated sWGA	Vector laboratories	B-1025S-5
NeutrAvidin Protein DyLight 800 conjugate	Thermo Fisher Scientific	22853
NeutrAvidin Protein DyLight 650 conjugate	Thermo Fisher Scientific	84607
FluoTag-X2 anti-ALFA conjugated with Alexa Fluor 647	NanoTag Biotechnologies	N1502

Supplementary Table 2. Complete sequence for cDNAs

Muc1 dCT	ATGACACCGGGCACCCAGTCTCCTTTCTTCCTGCTGCTGCTCCTCACAGTGCTTACAGTTGTTACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGAGAAAAGGAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCTACTGAGAAGAATGCTGATTACAAGGATGACGACGACCAGATCTTGGACATGGTCGCTGTGAGTATGACCAGCAGCGTACTCTCCAGCCACAGCCCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTCACTCTGGCCCCGGCCACGGAACCAGCTTCAGGTTCAGCTGCCACCTGGGGACAGGATGTCACCTCGGTCCCAGTCACCAGGCCAGCCCTGGGCTCCACCACCCCGCCAGCCCACGATGTCACCTCAGCCCCGGACAACAAGCCAGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG

	GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCC CCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCAC GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACC GCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCC CCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCAC GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACC GCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCC CCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCAC GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACC GCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCC CCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCAC GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACC GCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCC CCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCAC GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACC GCCCCCCCAGCCCATGGTGTCACCTCGGCCCCGGACAACAGGCCC GCCTTGGGCTCCACCGCCCCTCCAGTCCACAATGTCACCTCGGCC TCAGGCTCTGCATCAGGCTCAGCTTCTACTCTGGTGCACAACGGC ACCTCTGCCAGGGCTACCACAACCCCAGCCAGCAAGAGCACTCCA TTCTCAATTCCCAGCCACCACTCTGATACTCCTACCACCCTTGCCA GCCATAGCACCAAGACTGATGCCAGTAGCACTCACCATAGCTCGG TACCTCCTCTCACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTC TACTGGGGTCTCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCC AGTTTAATTCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAG AGCTGCAGAGAGACATTTCTGAAATGTTTTTGCAGATTTATAAAC AAGGGGGTTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGAT CTGTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCA ATGTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAA GCAGCCTCTCGATATAACCTGACGATCTCAGACGTCAGCGTGAGT GATGTGCCATTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAG GCTGGGGCATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCT GGCCATTGTCTATCTCATTGCCTTGGCTGTCTGTCAGTGC
Muc1 dCT-mOxGFP	ATGACACCGGGCACCCAGTCTCCTTTCTTCCTGCTGCTGCTCCTCA CAGTGCTTACAGTTGTTACAGGTTCTGGTCATGCAAGCTCTACCCC AGGTGGAGAAAAGGAGACTTCGGCTACCCAGAGAAGTTCAGTGC CCAGCTCTACTGAGAAGAATGCTGATTACAAGGATGACGACGAC CAGATCTTGGACATGGTCGCTGTGAGTATGACCAGCAGCGTACTC TCCAGCCACAGCCCCGGTTCAGGCTCCTCCACCACTCAGGGACAG GATGTCACTCTGGCCCCGGCCACGGAACCAGCTTCAGGTTCAGCT GCCACCTGGGGACAGGATGTCACCTCGGTCCCAGTCACCAGGCCA GCCCTGGGCTCCACCACCCCGCCAGCCCACGATGTCACCTCAGCC CCGGACAACAAGCCAGCCCCGGGCTCCACCGCCCCCCCAGCCCAC

	GGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCG

	GCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCACGGTGTCACCTCGGCCCCGGACACCAGGCCGGCCCCGGGCTCCACCGCCCCCCCAGCCCATGGTGTCACCTCGGCCCCGGACAACAGGCCCGCCTTGGGCTCCACCGCCCCTCCAGTCCACAATGTCACCTCGGCCTCAGGCTCTGCATCAGGCTCAGCTATGGTGTCCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCTCCGTGCGGGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATCAGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGAGCTTCTCCCGCTACCCCGACCACATGAAGCGCCACGACTTCTTCAAGAGCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTCCTTCAAGGACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACTCCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACGTGGAGGACGGCTCCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGTCCACCCAGTCCAAGCTGTCCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTTCTGGAATTCGTGACCGCCGCCGGGATCacTCACGGCATGGACGAGCTGTATAAGGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCTGCCAGGGCTACCACAACCCCAGCCAGCAAGAGCACTCCATTCTCAATTCCCAGCCACCACTCTGATACTCCTACCACCCTTGCCAGCCATAGCACCAAGACTGATGCCAGTAGCACTCACCATAGCTCGGTACCTCCTCTCACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACTGGGGTCTCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCCAGTTTAATTCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAGCTGCAGAGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAAGGGGGTTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCTGTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAATGTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCAGCCTCTCGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGATGTGCCATTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGCTGGGGCATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATTGTCTATCTCATTGCCTTGGCTGTCTGTCAGTGC
GCNT1	ATGCTGAGGACGTTGCTGCGAAGGAGACTTTTTTCTTATCCCACCAAATACTACTTTATGGTTCTTGTTTTATCCCTAATCACCTTCTCCGTTTTAAGGATTCATCAAAAGCCTGAATTTGTAAGTGTCAGACACTTGGAGCTTGCTGGGGAGAATCCTAGTAGTGATATTAATTGCACCAAAGTTTTACAGGGTGATGTAAATGAAATCCAAAAGGTAAAGCTTGA

	GATCCTAACAGTGAAATTTAAAAAGCGCCCTCGGTGGACACCTGACGACTATATAAACATGACCAGTGACTGTTCTTCTTTCATCAAGAGACGCAAATATATTGTAGAACCCCTTAGTAAAGAAGAGGCGGAGTTTCCAATAGCATATTCTATAGTGGTTCATCACAAGATTGAAATGCTTGACAGGCTGCTGAGGGCCATCTATATGCCTCAGAATTTCTATTGCATTCATGTGGACACAAAATCCGAGGATTCCTATTTAGCTGCAGTGATGGGCATCGCTTCCTGTTTTAGTAATGTCTTTGTGGCCAGCCGATTGGAGAGTGTGGTTTATGCATCGTGGAGCCGGGTTCAGGCTGACCTCAACTGCATGAAGGATCTCTATGCAATGAGTGCAAACTGGAAGTACTTGATAAATCTTTGTGGTATGGATTTTCCCATTAAAACCAACCTAGAAATTGTCAGGAAGCTCAAGTTGTTAATGGGAGAAAACAACCTGGAAACGGAGAGGATGCCATCCCATAAAGAAGAAAGGTGGAAGAAGCGGTATGAGGTCGTTAATGGAAAGCTGACAAACACAGGGACTGTCAAAATGCTTCCTCCACTCGAAACACCTCTCTTTTCTGGCAGTGCCTACTTCGTGGTCAGTAGGGAGTATGTGGGGTATGTACTACAGAATGAAAAAATCCAAAAGTTGATGGAGTGGGCACAAGACACATACAGCCCTGATGAGTATCTCTGGGCCACCATCCAAAGGATTCCTGAAGTCCCGGGCTCACTCCCTGCCAGCCATAAGTATGATCTGTCTGACATGCAAGCAGTTGCCAGGTTTGTCAAGTGGCAGTACTTTGAGGGTGATGTTTCCAAGGGTGCTCCCTACCCGCCCTGCGATGGAGTCCATGTGCGCTCAGTGTGCATTTTCGGAGCTGGTGACTTGAACTGGATGCTGCGCAAACACCACTTGTTTGCCAATAAGTTTGACGTGGATGTTGACCTCTTTGCCATCCAGTGTTTGGATGAGCATTTGAGACACAAAGCTTTGGAGACATTAAAACACTGA
LGALS3	AATTCTATGGCAGACAATTTTTCGCTCCATGATGCGTTATCTGGGTCTGGAAACCCAAACCCTCAAGGATGGCCTGGCGCATGGGGGAACCAGCCTGCTGGGGCAGGGGGCTACCCAGGGGCTTCCTATCCTGGGGCCTACCCCGGGCAGGCACCCCCAGGGGCTTATCCTGGACAGGCACCTCCAGGCGCCTACCCTGGAGCACCTGGAGCTTATCCCGGAGCACCTGCACCTGGAGTCTACCCAGGGCCACCCAGCGGCCCTGGGGCCTACCCATCTTCTGGACAGCCAAGTGCCACCGGAGCCTACCCTGCCACTGGCCCCTATGGCGCCCCTGCTGGGCCACTGATTGTGCCTTATAACCTGCCTTTGCCTGGGGGAGTGGTGCCTCGCATGCTGATAACAATTCTGGGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGATTTCCAAAGAGGGAATGATGTTGCCTTCCACTTTAACCCACGCTTCAATGAGAACAACAGGAGAGTCATTGTTTGCAATACAAAGCTGGATAATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCCCATTTGAAAGTGGGAAACCATTCAAAATACAAGTACTGGTTGAACCTGACCACTTCAAGGTTGCAGTGAATGATGCTCACTTGTTGCAGTACAATCATCGGGTTAAAAAACTCAATGAAATCAGCAAACTGGGAATTTCTGGTGACATAGACCTCACCAGTGCTTCATATACCATGATATAA
HER2	ATGGAGCTGGCGGCCTTGTGCCGCTGGGGGCTCCTCCTCGCCCTCTTGCCCCCCGGAGCCGCGAGCACCCAAGTGTGCACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACCTCTACCAGGGCTGCCAGGTGGTGCAGGGAAACCTGGAACTCACCTACCTGCCCACCAATGCCAGCCTGTCCTTCCTGCA

	GGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCACTGCAGAGGCTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACAACTATGCCCTGGCCGTGCTAGACAATGGAGACCCGCTGAACAATACCACCCCTGTCACAGGGGCCTCCCCAGGAGGCCTGCGGGAGCTGCAGCTTCGAAGCCTCACAGAGATCTTGAAAGGAGGGGTCTTGATCCAGCGGAACCCCCAGCTCTGCTACCAGGACACGATTTTGTGGAAGGACATCTTCCACAAGAACAACCAGCTGGCTCTCACACTGATAGACACCAACCGCTCTCGGGCCTGCCACCCCTGTTCTCCGATGTGTAAGGGCTCCCGCTGCTGGGGAGAGAGTTCTGAGGATTGTCAGAGCCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCAAGGGGCCACTGCCCACTGACTGCTGCCATGAGCAGTGTGCTGCCGGCTGCACGGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACCACAGTGGCATCTGTGAGCTGCACTGCCCAGCCCTGGTCACCTACAACACAGACACGTTTGAGTCCATGCCCAATCCCGAGGGCCGGTATACATTCGGCGCCAGCTGTGTGACTGCCTGTCCCTACAACTACCTTTCTACGGACGTGGGATCCTGCACCCTCGTCTGCCCCCTGCACAACCAAGAGGTGACAGCAGAGGATGGAACACAGCGGTGTGAGAAGTGCAGCAAGCCCTGTGCCCGAGTGTGCTATGGTCTGGGCATGGAGCACTTGCGAGAGGTGAGGGCAGTTACCAGTGCCAATATCCAGGAGTTTGCTGGCTGCAAGAAGATCTTTGGGAGCCTGGCATTTCTGCCGGAGAGCTTTGATGGGGACCCAGCCTCCAACACTGCCCCGCTCCAGCCAGAGCAGCTCCAAGTGTTTGAGACTCTGGAAGAGATCACAGGTTACCTATACATCTCAGCATGGCCGGACAGCCTGCCTGACCTCAGCGTCTTCCAGAACCTGCAAGTAATCCGGGGACGAATTCTGCACAATGGCGCCTACTCGCTGACCCTGCAAGGGCTGGGCATCAGCTGGCTGGGGCTGCGCTCACTGAGGGAACTGGGCAGTGGACTGGCCCTCATCCACCATAACACCCACCTCTGCTTCGTGCACACGGTGCCCTGGGACCAGCTCTTTCGGAACCCGCACCAAGCTCTGCTCCACACTGCCAACCGGCCAGAGGACGAGTGTGTGGGCGAGGGCCTGGCCTGCCACCAGCTGTGCGCCCGAGGGCACTGCTGGGGTCCAGGGCCCACCCAGTGTGTCAACTGCAGCCAGTTCCTTCGGGGCCAGGAGTGCGTGGAGGAATGCCGAGTACTGCAGGGGCTCCCCAGGGAGTATGTGAATGCCAGGCACTGTTTGCCGTGCCACCCTGAGTGTCAGCCCCAGAATGGCTCAGTGACCTGTTTTGGACCGGAGGCTGACCAGTGTGTGGCCTGTGCCCACTATAAGGACCCTCCCTTCTGCGTGGCCCGCTGCCCCAGCGGTGTGAAACCTGACCTCTCCTACATGCCCATCTGGAAGTTTCCAGATGAGGAGGGCGCATGCCAGCCTTGCCCCATCAACTGCACCCACTCCTGTGTGGACCTGGATGACAAGGGCTGCCCCGCCGAGCAGAGAGCCAGCCCTCTGACGTCCATCATCTCTGCGGTGGTTGGCATTCTGCTGGTCGTGGTCTTGGGGGTGGTCTTTGGGATCCTCATCAAGCGACGGCAGCAGAAGATCCGGAAGTACACGATGCGGAGACTGCTGCAGGAAACGGAGCTGGTGGAGCCGCTGACACCTAGCGGAGCGATGCCCAACCAGGCGCAGATGCGGATCCTGAAAGAGACGGAGCTGAGGAAGGTGAAGGTGCTTGGATCTGGCGCTTTTGGCACAGTCTACAAGGGCATCTGGATCCCTGATGGGGAGAATGTGAAAATTCCAGTGGCCATCAAAGTGTTGAGGGAAAACACATCCCCCAAAG

	CCAACAAAGAAATCTTAGACGAAGCATACGTGATGGCTGGTGTGGGCTCCCCATATGTCTCCCGCCTTCTGGGCATCTGCCTGACATCCACGGTGCAGCTGGTGACACAGCTTATGCCCTATGGCTGCCTCTTAGACCATGTCCGGGAAAACCGCGGACGCCTGGGCTCCCAGGACCTGCTGAACTGGTGTATGCAGATTGCCAAGGGGATGAGCTACCTGGAGGATGTGCGGCTCGTACACAGGGACTTGGCCGCTCGGAACGTGCTGGTCAAGAGTCCCAACCATGTCAAAATTACAGACTTCGGGCTGGCTCGGCTGCTGGACATTGACGAGACAGAGTACCATGCAGATGGGGGCAAGGTGCCCATCAAGTGGATGGCGCTGGAGTCCATTCTCCGCCGGCGGTTCACCCACCAGAGTGATGTGTGGAGTTATGGTGTGACTGTGTGGGAGCTGATGACTTTTGGGGCCAAACCTTACGATGGGATCCCAGCCCGGGAGATCCCTGACCTGCTGGAAAAGGGGGAGCGGCTGCCCCAGCCCCCCATCTGCACCATTGATGTCTACATGATCATGGTCAAATGTTGGATGATTGACTCTGAATGTCGGCCAAGATTCCGGGAGTTGGTGTCTGAATTCTCCCGCATGGCCAGGGACCCCCAGCGCTTTGTGGTCATCCAGAATGAGGACTTGGGCCCAGCCAGTCCCTTGGACAGCACCTTCTACCGCTCACTGCTGGAGGACGATGACATGGGGGACCTGGTGGATGCTGAGGAGTATCTGGTACCCCAGCAGGGCTTCTTCTGTCCAGACCCTGCCCCGGGCGCTGGGGGCATGGTCCACCACAGGCACCGCAGCTCATCTACCAGGAGTGGCGGTGGGGACCTGACACTAGGGCTGGAGCCCTCTGAAGAGGAGGCCCCCAGGTCTCCACTGGCACCCTCCGAAGGGGCTGGCTCCGATGTATTTGATGGTGACCTGGGAATGGGGGCAGCCAAGGGGCTGCAAAGCCTCCCCACACATGACCCCAGCCCTCTACAGCGGTACAGTGAGGACCCCACAGTACCCCTGCCCTCTGAGACTGATGGCTACGTTGCCCCCCTGACCTGCAGCCCCCAGCCTGAATATGTGAACCAGCCAGATGTTCGGCCCCAGCCCCCTTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCCGACCTGCTGGTGCCACTCTGGAAAGGCCCAAGACTCTCTCCCCAGGGAAGAATGGGGTCGTCAAAGACGTTTTTGCCTTTGGGGGTGCCGTGGAGAACCCCGAGTACTTGACACCCCAGGGAGGAGCTGCCCCTCAGCCCCACCCTCCTCCTGCCTTCAGCCCAGCCTTCGACAACCTCTATTACTGGGACCAGGACCCACCAGAGCGGGGGGCTCCACCCAGCACCTTCAAAGGGACACCTACGGCAGAGAACCCAGAGTACCTGGGTCTGGACGTGCCAGTGTGA
CD19	ATGCCTCCCCCACGACTGTTGTTTTTTCTCCTCTTTCTGACACCAATGGAGGTGCGCCCAGAAGAGCCTCTGGTGGTTAAGGTAGAAGAAGGGGATAATGCCGTACTCCAATGCCTTAAGGGGACATCAGATGGACCAACTCAACAGTTGACCTGGAGCAGGGAGTCCCCTCTCAAACCTTTTCTTAAGTTGTCACTTGGCCTTCCAGGGCTCGGTATCCATATGAGACCCCTCGCAATCTGGTTGTTCATCTTTAACGTCTCTCAGCAGATGGGAGGGTTTTACCTTTGCCAGCCAGGACCACCTAGCGAAAAGGCTTGGCAACCAGGTTGGACTGTCAATGTTGAGGGAAGCGGTGAGTTGTTTCGCTGGAACGTAAGCGACCTGGGGGGGCTCGGATGTGGATTGAAAAATCGATCCTCCGAAGGGCCTTCTTCCCCTTCAGGCAAGTTGATGTCCCCCAAACTGTATGTATGGGCCAAGGATCGGCCCGAGATTTGGGAAGGAGAACCCCCATGCTTGCCTCCAAGAGACAGTCTTA

	ATCAGTCCCTTTCACAGGACTTGACTATGGCCCCAGGTAGTACCTTGTGGCTGTCATGCGGTGTGCCCCCTGATAGTGTGTCTCGGGGTCCTCTGAGCTGGACCCACGTCCATCCCAAAGGACCAAAATCCCTTCTTTCACTCGAATTGAAAGACGACCGCCCTGCACGCGACATGTGGGTGATGGAGACTGGCCTTTTGCTCCCCCGAGCCACAGCTCAGGATGCAGGAAAGTACTACTGTCATCGAGGTAACCTGACTATGTCCTTTCACCTGGAAATCACTGCACGCCCAGTGCTTTGGCATTGGTTGCTCAGAACAGGAGGCTGGAAGGTTAGCGCAGTCACATTGGCTTATCTGATCTTCTGTCTTTGTAGCCTTGTCGGAATACTGCACCTGCAGCGAGCCCTTGTGCTCAGACGCAAGCGGAAGAGAATGACTGATCCAACAAGAAGGTTCTGA
Complete Sequence of HER2 CAR[8] FRP5 scFV CD8α hinge CD28 TM and co-stimulatory domain CD3ζ signaling domain P2A mTagBFP2	ATGGACTGGATATGGAGAATATTGTTTCTGGTGGGCGCTGCTACCGGGGCTCATAGTCAAGTTCAACTTCAACAAAGTGGGCCAGAACTCAAGAAACCAGGGGAAACTGTGAAAATTTCATGTAAAGCTTCAGGATATCCATTTACAAATTATGGGATGAATTGGGTCAAGCAAGCTCCCGGACAAGGTCTCAAATGGATGGGGTGGATAAATACGTCCACAGGTGAATCCACATTTGCGGATGACTTTAAAGGACGGTTTGATTTTAGTCTCGAGACTTCTGCTAACACTGCTTATCTCCAAATTAATAATCTGAAATCTGAAGATTCCGCAACATATTTCTGTGCACGGTGGGAAGTCTATCATGGTTATGTCCCTTATTGGGGACAAGGTACAACTGTAACTGTATCTTCCGGTGGGGGGGGAAGTGGAGGAGGCGGCAGTGGTGGTGGCGGGTCTGATATACAACTCACGCAATCTCATAAATTCCTTTCTACGTCCGTTGGAGATAGAGTCAGCATTACGTGTAAGGCTTCCCAAGATGTCTATAATGCAGTCGCATGGTACCAACAGAAACCAGGGCAAAGTCCTAAATTGCTTATATATTCTGCTTCTTCCAGGTATACTGGTGTCCCTTCCCGCTTTACGGGGTCCGGATCTGGGCCTGATTTTACGTTTACTATTTCATCCGTCCAAGCGGAAGATCTCGCTGTCTATTTCTGTCAACAGCATTTTCGTACCCCTTTTACATTTGGAAGTGGAACGAAACTCGAGATAAAAGCACTTAGTAATTCCATTATGTATTTTAGCCATTTTGTCCCAGTCTTCCTTCCAGCAAAACCTACTACAACACCAGCGCCACGCCCACCAACGCCTGCACCTACGATAGCATCTCAACCACTCAGTCTCCGACCAGAAGCGTCTAGGCCAGCGGCCGGCGGCGCGGTCCATACAAGAGGGCTTGATAAACCATTTTGGGTACTCGTGGTTGTAGGTGGTGTTTTGGCTTGCTATTCCCTGCTTGTTACAGTCGCGTTTATTATTTTCTGGGTTCGTTCTAAAAGATCTCGCCTTTTGCATTCTGATTATATGAATATGACTCCCCGCAGACCTGGGCCGACTAGAAAACATTATCAACCGTACGCTCCCCCGCGGGATTTTGCTGCATATAGAAGCCGGGTCAAATTTTCACGTTCCGCTGATGCACCCGCATATCAACAAGGACAAAATCAACTTTATAATGAACTCAATCTCGGGCGGCGCGAAGAGTATGATGTTCTCGATAAAAGGCGGGGCCGAGATCCCGAAATGGGTGGGAAACCGCGGAGAAAGAATCCTCAAGAGGGGCTTTACAATGAGTTGCAAAAGGATAAAATGGCGGAAGCTTATTCTGAAATTGGGATGAAAGGAGAACGCCGCAGAGGGAAAGGTCATGATGGGCTCTATCAAGGGCTCAGCACGGCAACTAAAGATACTTATGATGCTCTTCATATGCAAGCTCTCCCACCGCGAGCTTCCGCTACAAACTTTTCCCTTCTCAAACAGGCTGGAGATGTAGAGGAGAATCCAGGGCC

	GATGGTCAGCAAAGGAGAGGAACTTATAAAGGAAAATATGCATATGAAATTGTACATGGAAGGTACAGTCGATAATCACCATTTTAAGTGTACCTCTGAAGGAGAGGGGAAACCTTATGAAGGAACTCAAACTATGAGGATTAAAGTCGTGGAAGGTGGGCCACTTCCATTCGCGTTTGATATTCTCGCAACAAGTTTTCTGTATGGTTCCAAAACCTTTATTAATCATACGCAAGGAATCCCTGATTTCTTTAAACAATCATTTCCCGAAGGCTTTACCTGGGAACGCGTGACGACGTATGAGGATGGTGGAGTCTTGACTGCCACTCAAGATACTTCACTGCAAGATGGGTGTCTGATTTATAATGTGAAAATTCGCGGCGTCAATTTTACCTCTAATGGGCCAGTCATGCAAAAGAAGACTTTGGGTTGGGAAGCATTTACAGAAACCTTGTATCCAGCGGATGGTGGACTTGAGGGGAGAAATGATATGGCACTCAAACTGGTTGGCGGAAGTCACCTCATAGCTAATGCTAAAACGACTTACCGCTCTAAGAAGCCGGCCAAGAATCTGAAAATGCCCGGTGTGTATTACGTTGATTATCGGCTCGAGCGGATTAAAGAAGCGAATAATGAAACATATGTGGAACAACATGAAGTTGCCGTCGCACGGTATTGTGATCTGCCATCAAAGCTCGGCCATAAACTGAAC
Cytosolic mScarlet-I	ATGACCGGATTGCAGAAGAAGCTGGAGGAGCTAGAGCTTGATATGGTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGTTCAAGGTGCACATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCTCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAGGGCCTTCATCAAGCACCCCGCCGACATCCCCGACTACTATAAGCAGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGCCGTGACCGTGACCCAGGACACCTCCCTGGAGGACGGCACCCTGATCTACAAGGTGAAGCTCCGCGGCACCAACTTCCCTCCTGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAAGCGTCCACCGAGCGGTTGTACCCCGAGGACGGCGTGCTGAAGGGCGACATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGCTACCTGGCGGACTTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTCGACCGCAAGTTGGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGCTCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACTGA
StcE Δ35 (without signal peptide)	GCTAGCGCTGATAATAATTCAGCCATTTATTTCAATACCTCCCAGCCTATAAATGATCTGCAGGGTTCGTTGGCCGCAGAGGTGAAATTTGCACAAAGCCAGATTTTACCCGCCCATCCTAAAGAAGGGGATAGTCAACCACATCTGACCAGCCTGCGGAAAAGTCTGCTGCTTGTCCGTCCGGTGAAAGCTGATGATAAAACACCTGTTCAGGTGGAAGCCCGCGATGATAATAATAAAATTCTCGGTACGTTAACCCTTTATCCTCCTTCATCACTACCGGATACAATCTACCATCTGGATGGTGTTCCGGAAGGTGGTATCGATTTCACACCTCATAATGGAACGAAAAAGATCATTAATACGGTGGCTGAAGTAAACAAACTCAGTGATGCCAGCGGGAGTTCTATTCATAGCCATCTAACAAATAATGCACTGGTGGAGATCCATACTGCAAATGGTCGTTGGGTAAGAGACATTTATCTGCCGCAGGGACCCGACCTTGAAGGTAAGATGGTTCGCTTTGTTTCGTCTGCAGGCTATAGTTCAACGGTTTTTTATGGTGATCGAAAAGTCACACTCTCGG

	TGGGTAACACTCTTCTGTTCAAATATGTAAATGGTCAGTGGTTCCGCTCCGGTGAACTGGAGAATAATCGAATCACTTATGCTCAGCATATTTGGAGTGCTGAACTGCCTGCGCACTGGATCGTGCCTGGTTTAAACTTGGTGATTAAACAGGGCAATCTGAGCGGTCGCCTAAATGATATCAAGATTGGAGCACCGGGTGAGCTGTTGTTGCATACAATTGATATCGGGATGTTGACCACTCCCCGGGATCGCTTTGATTTTGCCAAAGACAAAGAAGCACATAGGGAATATTTCCAGACCATTCCTGTAAGTCGTATGATTGTTAATAATTATGCGCCTCTACACCTAAAGGAAGTTATGTTACCAACCGGAGAGTTATTGACAGATATGGATCCAGGAAATGGTGGGTGGCATAGTGGTACAATGCGTCAAAGAATAGGTAAAGAATTGGTTTCGCATGGCATTGATAATGCTAACTATGGTTTAAATAGTACCGCAGGCTTAGGGGAGAATAGTCATCCATATGTAGTTGCGCAATTAGCGGCACATAATAGCCGCGGTAATTATGCTAATGGCATCCAGGTTCATGGTGGCTCCGGAGGTGGGGGAATTGTTACTTTAGATTCCACATTGGGGAATGAGTTCAGTCATGAAGTTGGTCATAATTATGGTCTTGGTCATTATGTAGATGGTTTCAAGGGTTCTGTACATCGTAGTGCAGAAAATAACAACTCAACTTGGGGATGGGATGGTGATAAAAAACGGTTTATTCCTAACTTTTATCCGTCTCAAACAAATGAAAAGAGTTGTCTGAATAATCAGTGTCAAGAACCGTTTGATGGACACAAATTTGGTTTTGACGCCATGGCGGGAGGCAGCCCTTTCTCTGCTGCAAACCGTTTCACAATGTATACTCCGAATTCATCGGCTATCATCCAGCGTTTTTTTGAAAATAAAGCTGTGTTCGATAGCCGTTCCTCCACCGGCTTCAGCAAGTGGAATGCAGATACGCAGGAAATGGAACCGTATGAACACACCATTGACCGTGCGGAGCAGATTACGGCTTCAGTCAATGAGCTAAGTGAAAGCAAAATGGCTGAGCTGATGGCAGAGTACGCTGTCGTCAAAGTGCATATGTGGAACGGTAACTGGACAAGAAACATCTATATCCCTACAGCCTCCGCAGATAATAGAGGCAGTATCCTGACCATCAACCATGAGGCCGGTTATAATAGTTATCTGTTTATAAATGGTGACGAAAAGGTCGTTTCCCAGGGGTATAAAAAGAGCTTTGTTTCCGATGGTCAGTTCTGGAAAGAACGTGATGTGGTTGATACTCGTGAAGCGCGTAAGCCAGAGCAGTTTGGTGTTCCTGTGACGACCCTGGTGGGGTATTACGATCCGGAAGGCACGCTGTCAAGCTACATCTATCCTGCGATGTATGGTGCCTATGGCTTCACTTATTCCGATGATAGTCAGAATCTATCCGATAACGACTGCCAGCTGCAGGTGGATACGAAAGAAGGGCAGTTGCGATTCAGACTGGCTAATCACCGGGCTAACAACACTGTAATGAATAAGTTCCATATTAACGTGCCAACAGAAAGTCAGCCCACACAGGCCACATTGGTTTGCAATAACAAGATACTGGATACCAAATCGCTCACACCTGCGCCAGAAGGACTTACCTATACTGTAAATGGGCAGGCACTTCCAGCAAAAGAAAACGAGGGATGCATCGTGTCCGTGAATTCAGGTAAACGTTACTGTTTGCCGGTTGGTCAACGGTCAGGATATAGCCTTCCTGACTGGATTGTTGGGCAGGAAGTCTATGTCGACAGCGGGGCTAAAGCGAAAGTGCTGCTTTCTGACTGGGATAACCTGTCCTATAACAGGATTGGTGAGTTTGTAGGTAATGTGAACCCAGCTGATATGAAAAAAGTTAAAGCCTGGAACGGACAGTATTTGGACTTCAGTAAACCTAGGTCAATGAGGGTTGTATATAAA

StcE ΔX409[4]	GCTAGCGCTGATAATAATTCAGCCATTTATTTCAATACCTCCCAGCCTATAAATGATCTGCAGGGTTCGTTGGCCGCAGAGGTGAAATTTGCACAAAGCCAGATTTTACCCGCCCATCCTAAAGAAGGGGATAGTCAACCACATCTGACCAGCCTGCGGAAAAGTCTGCTGCTTGTCCGTCCGGTGAAAGCTGATGATAAAACACCTGTTCAGGTGGAAGCCCGCGATGATAATAATAAAATTCTCGGTACGTTAACCCTTTATCCTCCTTCATCACTACCGGATACAATCTACCATCTGGATGGTGTTCCGGAAGGTGGTATCGATTTCACACCTCATAATGGAACGAAAAAGATCATTAATACGGTGGCTGAAGTAAACAAACTCAGTGATGCCAGCGGGAGTTCTATTCATAGCCATCTAACAAATAATGCACTGGTGGAGATCCATACTGCAAATGGTCGTTGGGTAAGAGACATTTATCTGCCGCAGGGACCCGACCTTGAAGGTAAGATGGTTCGCTTTGTTTCGTCTGCAGGCTATAGTTCAACGGTTTTTTATGGTGATCGAAAAGTCACACTCTCGGTGGGTAACACTCTTCTGTTCAAATATGTAAATGGTCAGTGGTTCCGCTCCGGTGAACTGGAGAATAATCGAATCACTTATGCTCAGCATATTTGGAGTGCTGAACTGCCTGCGCACTGGATCGTGCCTGGTTTAAACTTGGTGATTAAACAGGGCAATCTGAGCGGTCGCCTAAATGATATCAAGATTGGAGCACCGGGTGAGCTGTTGTTGCATACAATTGATATCGGGATGTTGACCACTCCCCGGGATCGCTTTGATTTTGCCAAAGACAAAGAAGCACATAGGGAATATTTCCAGACCATTCCTGTAAGTCGTATGATTGTTAATAATTATGCGCCTCTACACCTAAAGGAAGTTATGTTACCAACCGGAGAGTTATTGACAGATATGGATCCAGGAAATGGTGGGTGGCATAGTGGTACAATGCGTCAAAGAATAGGTAAAGAATTGGTTTCGCATGGCATTGATAATGCTAACTATGGTTTAAATAGTACCGCAGGCTTAGGGGAGAATAGTCATCCATATGTAGTTGCGCAATTAGCGGCACATAATAGCCGCGGTAATTATGCTAATGGCATCCAGGTTCATGGTGGCTCCGGAGGTGGGGGAATTGTTACTTTAGATTCCACATTGGGGAATGAGTTCAGTCATGAAGTTGGTCATAATTATGGTCTTGGTCATTATGTAGATGGTTTCAAGGGTTCTGTACATCGTAGTGCAGAAAATAACAACTCAACTTGGGGATGGGATGGTGATAAAAAACGGTTTATTCCTAACTTTTATCCGTCTCAAACAAATGAAAAGAGTTGTCTGAATAATCAGTGTCAAGAACCGTTTGATGGACACAAATTTGGTTTTGACGCCATGGCGGGAGGCAGCCCTTTCTCTGCTGCAAACCGTTTCACAATGTATACTCCGAATTCATCGGCTATCATCCAGCGTTTTTTTGAAAATAAAGCTGTGTTCGATAGCCGTTCCTCCACCGGCTTCAGCAAGTGGAATGCAGATACGCAGGAAATGGAACCGTATGAACACACCATTGACCGTGCGGAGCAGATTACGGCTTCAGTCAATGAGCTAAGTGAAAGCAAAATGGCTGAGCTGATGGCAGAGTACGCTGTCGTCAAAGTGCATATGTGGAACGGTAACTGGACAAGAAACATCTATATCCCTACAGCCTCCGCAGATAATAGAGGCAGTATCCTGACCATCAACCATGAGGCCGGTTATAATAGTTATCTGTTTATAAATGGTGACGAAAAGGTCGTTTCCCAGGGGTATAAAAAGAGCTTTGTTTCCGATGGTCAGTTCTGGAAAGAACGTGATGTGGTTGATACTCGTGAAGCGCGTAAGCCAGAGCAGTTTGGTGTTCCTGTGACGACCCTGGTGGGGTATTACGATCCGGAAGGCACGCTGTCAAGCTACATCTATCCTGCGATGTATGGTGCCTATGGCTTCACTTATTCCGATGATAGTCAGAATC

	TATCCGATAACGACTGCCAGCTGCAGGTGGATACGAAAGAAGGGCAGTTGCGATTCAGACTGGCTAATCACCGGGCTAACAACACTGTAATGAATAAGTTCCATATTAACGTGCCAACAGAAAGTCAGCCCACACAGGCCACATTGGTTTGCAATAACAAGATACTGGATACCAAATCGCTCACACCTGCGCCAGAAGGACTTACCTATACTGTAAAT
ST Sialidase (*S. typhimurium*)	ACCGTAGAGAAGTCAGTGGTGTTCAAAGCCGAAGGCGAGCATTTTACCGATCAGAAAGGAAATACCATTGTGGGTTCTGGGTCGGGTGGGACTACAAAATATTTCCGTATCCCGGCAATGTGTACCACATCAAAGGGTACGATCGTTGTTTTCGCTGACGCCCGCCATAATACTGCGTCTGATCAAAGTTTCATCGATACAGCCGCCGCACGTAGCACGGACGGCGGGAAAACTTGGAATAAAAAAATCGCTATCTACAATGACCGCGTCAATAGCAAGCTGTCTCGTGTTATGGATCCTACTTGCATTGTCGCTAATATTCAGGGTCGCGAGACCATCTTAGTTATGGTAGGAAAGTGGAACAACAATGACAAAACTTGGGGTGCTTATCGCGACAAAGCCCCTGATACTGATTGGGATCTGGTTTTATACAAGTCAACCGACGACGGAGTAACTTTCAGCAAGGTTGAAACTAATATTCACGATATCGTAACCAAAAATGGAACAATTTCAGCAATGTTAGGTGGCGTAGGGAGCGGATTACAACTTAACGACGGAAAACTGGTCTTCCCGGTACAAATGGTCCGCACAAAAAATATCACGACTGTCTTGAACACCTCCTTTATCTACTCAACCGACGGGATCACCTGGAGTTTACCTTCTGGATACTGCGAGGGTTTCGGCTCCGAAAACAACATCATTGAATTCAATGCGTCTTTGGTCAACAACATCCGCAATTCAGGGTTGCGCCGTTCGTTTGAGACAAAGGACTTCGGGAAGACCTGGACGGAGTTTCCGCCGATGGACAAAAAAGTGGACAATCGTAATCATGGCGTCCAAGGCAGCACTATTACGATTCCTTCAGGAAACAAACTGGTGGCTGCCCACAGTTCTGCCCAGAATAAAAACAATGATTACACTCGCTCTGATATTTCACTGTACGCTCATAACCTTTATTCAGGAGAGGTTAAATTGATTGACGCATTTTACCCGAAGGTCGGCAACGCATCTGGGGCCGGGTATAGCTGTTTGAGCTACCGCAAAAACGTTGACAAGGAGACCTTGTATGTAGTGTACGAAGCAAATGGCTCTATTGAGTTCCAAGACCTGTCGCGCCATCTGCCCGTCATTAAGTCGTACAAC
EE leucine zipper [10]	ATGGACCCTGATCTTGAGATTGAAGCTGCCTTCCTTGAACGGGAGAACACGGCCTTGGAAACGAGAGTGGCGGAGTTAAGACAAAGAGTCCAGCGCCTTCGTAACCGCGTAAGCCAGTATCGGACCCGGTACGGACCCCTTGGCGGGGGAAAG
RR leucine zipper [10]	ATGGACCCTGATCTCGAAATTCGCGCGGCCTTCCTCAGGCAGCGAAATACCGCTTTGAGAACGGAAGTCGCTGAGCTCGAACAAGAGGTCCAGAGACTGGAGAACGAAGTGAGCCAATATGAAACACGATATGGCCCCCTCGGCGGCGGAAAG
StcE ΔX409-35 aa Linker-EE Zip	GCTAGCGCTGATAATAATTCAGCCATTTATTTCAATACCTCCCAGCCTATAAATGATCTGCAGGGTTCGTTGGCCGCAGAGGTGAAATTTGCACAAAGCCAGATTTTACCCGCCCATCCTAAAGAAGGGGATAGTCAACCACATCTGACCAGCCTGCGGAAAAGTCTGCTGCTTGTCCGTCCGGTGAAAGCTGATGATAAAACACCTGTTCAGGTGGAAGCCCGCGATGATAATAATAAAATTCTCGGTACGTTAACCCTTTATCCTCCTTCATCACTACCGGATACAATCTACCATCTGGATGGTGTTCCGGAAG

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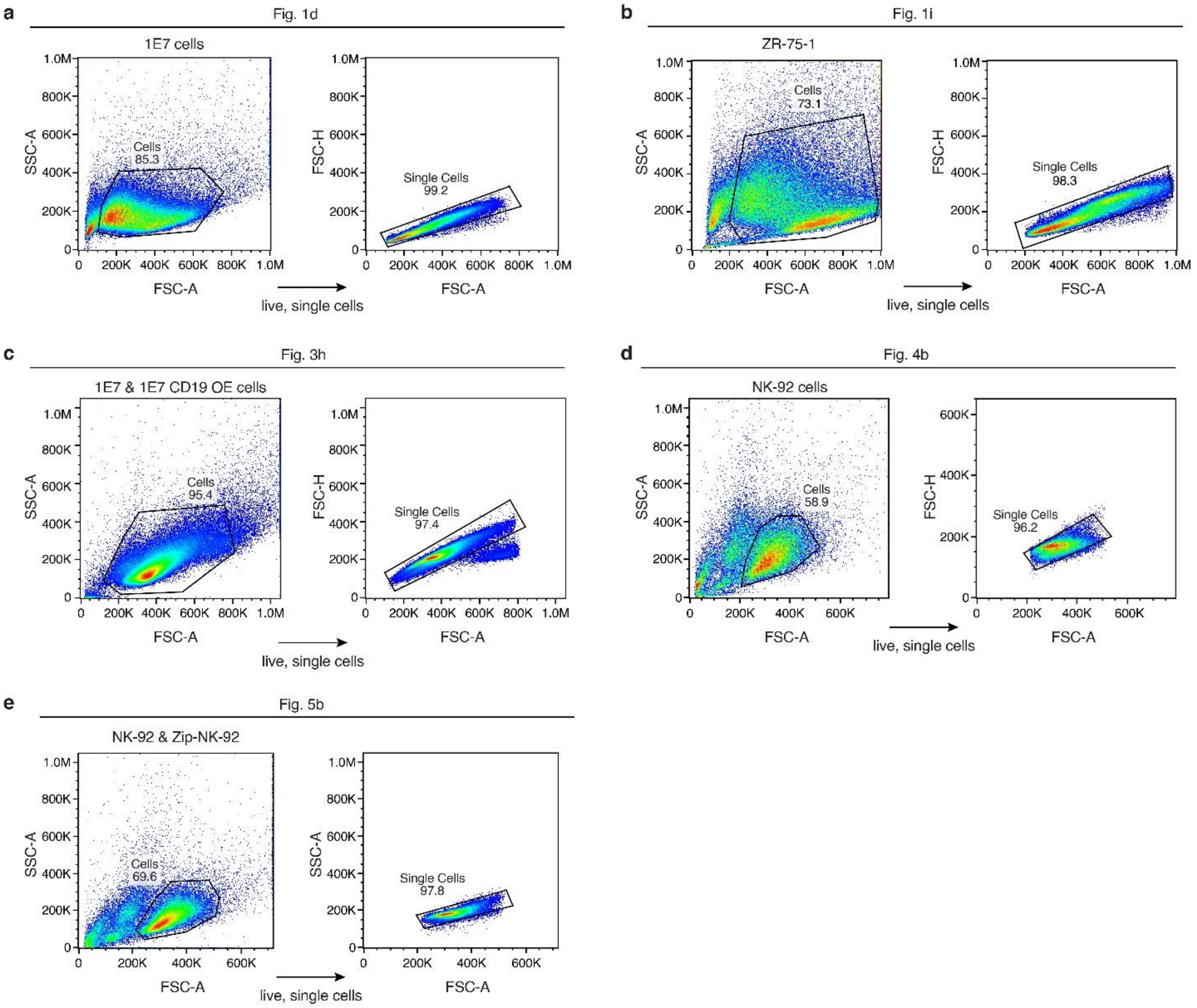
ST Sialidase-35 aa Linker-EE Zip	ACCGTAGAGAAGTCAGTGGTGTTCAAAGCCGAAGGCGAGCATTTTACCGATCAGAAAGGAAATACCATTGTGGGTTCTGGGTCGGGTGGGACTACAAAATATTTCCGTATCCCGGCAATGTGTACCACATCAAAGGGTACGATCGTTGTTTTCGCTGACGCCCGCCATAATACTGCGTCTGATCAAAGTTTCATCGATACAGCCGCCGCACGTAGCACGGACGGCGGGAAAACTTGGAATAAAAAAATCGCTATCTACAATGACCGCGTCAATAGCAAGCTGTCTCGTGTTATGGATCCTACTTGCATTGTCGCTAATATTCAGGGTCGCGAGACCATCTTAGTTATGGTAGGAAAGTGGAACAACAATGACAAAACTTGGGGTGCTTATCGCGACAAAGCCCCTGATACTGATTGGGATCTGGTTTTATACAAGTCAACCGACGACGGAGTAACTTTCAGCAAGGTTGAAACTAATATTCACGATATCGTAACCAAAAATGGAACAATTTCAGCAATGTTAGGTGGCGTAGGGAGCGGATTACAACTTAACGACGGAAAACTGGTCTTCCCGGTACAAATGGTCCGCACAAAAAATATCACGACTGTCTTGAACACCTCCTTTATCTACTCAACCGACGGGATCACCTGGAGTTTACCTTCTGGATACTGCGAGGGTTTCGGCTCCGAAAACAACATCATTGAATTCAATGCGTCTTTGGTCAACAACATCCGCAATTCAGGGTTGCGCCGTTCGTTTGAGACAAAGGACTTCGGGAAGACCTGGACGGAGTTTCCGCCGATGGACAAAAAAGTGGACAATCGTAATCATGGCGTCCAAGGCAGCACTATTACGATTCCTTCAGGAAACAAACTGGTGGCTGCCCACAGTTCTGCCCAGAATAAAAACAATGATTACACTCGCTCTGATATTTCACTGTACGCTCATAACCTTTATTCAGGAGAGGTTAAATTGATTGACGCATTTTACCCGAAGGTCGGCAACGCATCTGGGGCCGGGTATAGCTGTTTGAGCTACCGCAAAAACGTTGACAAGGAGACCTTGTATGTAGTGTACGAAGCAAATGGCTCTATTGAGTTCCAAGACCTGTCGCGCCATCTGCCCGTCATTAAGTCGTACAACGGTGGAGGCGGTTCTGGCGGTGGCGGCTCAGGTGGAGGTGGCTCTGGCGGAGGAGGTAGCGGAGGTGGCGGTAGCGGCGGAGGAGGTTCTGGTGGAGGAGGTAGCATGGACCCTGATCTTGAGATTGAAGCTGCCTTCCTTGAACGGGAGAACACGGCCTTGGAAACGAGAGTGGCGGAGTTAAGACAAAGAGTCCAGCGCCTTCGTAACCGCGTAAGCCAGTATCGGACCCGGTACGGACCCCTTGGCGGGGGAAAG
Surface display leucine zipper for Zip-NK-92 IgK leader ALFA Tag RR Zip	ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACTCAAGATTGGAGGAGGAATTGCGGAGACGATTGACCGAGCCAATGGACCCTGATCTCGAAATTCGCGCGGCCTTCCTCAGGCAGCGAAATACCGCTTTGAGAACGGAAGTCGCTGAGCTCGAACAAGAGGTCCAGAGACTGGAGAACGAAGTGAGCCAATATGAAACACGATATGGCCCCCTCGGCGGCGGAAAGTTGGAAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTGCCACACTCCTTGCCCTTTAAGGTGGTGGTGATCTCAGCCATCCTGGCCCTGGTGGTGCTCACCATCATCTCCCTTATCATCCTCATCATGCTTTGGCAGAAGAAGCCACGTACT

Myc Tag PDGFRβ TM sfGFP	AGTGGTTCTGGTTCTATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAA*

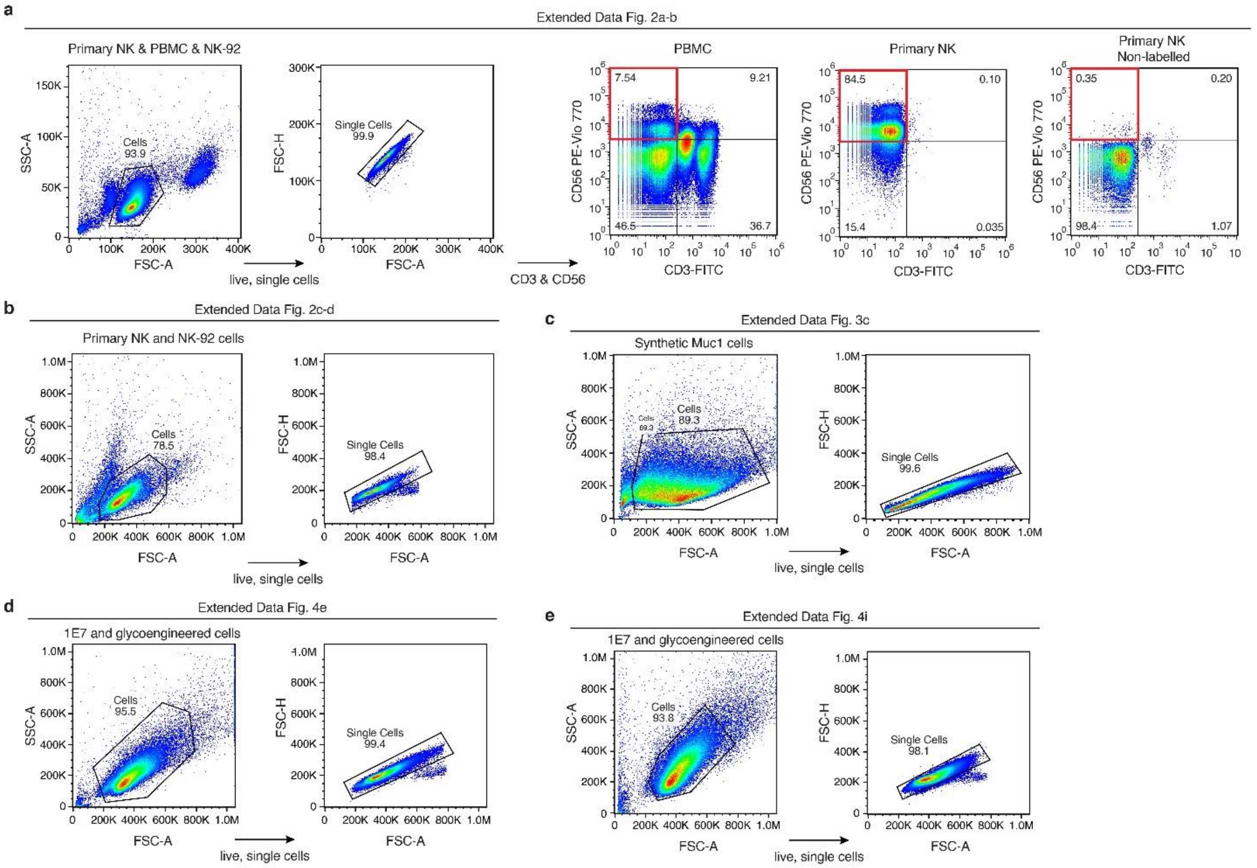
Supplementary Table 3. PCR primer sequence and CRISPR RNA sequence for cloning

PCR primers for human *HER2*	5’- TAATCTAGACCCAAGCTTGGGGCAG -3’ 5’- GCTGTCGACGAGCTCGGTACCAAGCTT-3’
PCR primers for human *LSGALS3*	5’- TAAGCGGATCCGAATTCTATGGCAGACAATT-3’ 5’-TGCTTTGTACATTATATCATGGTATATGAAGC-3’
CRISPR RNA and HDR template for *C1GALT1* KO	crRNA: 5’-GTAAAGCAGGGCTACATGAG-3’ HDR template: 5’-CTTTGGGAGAAGATTTAAGCCTTATGTAAAGCAGGGCTACTAAGAATTCAGTGGAGGAGCAGGATATGTACTAAGCAAAGAAGCCTTGA-3’
PCR primers for screening *C1GALT1*	5’-GGAGGATAATAGTTGTAATTCCAGTACCAAAAC-3’ 5’-TCAAAACCTAGAGAAAAAGGCCAAACAC-3’
PCR primers for screening *GNE*	5’- AGTGGTTAAGGACTTGAAACTG-3’ 5’ -TCTACTAAGCGGCATCATTG-3’
Sequencing primer for *C1GALT1*	5’-ACACGTCAAAGCTACTTGG-3’
Sequencing primer for *GNE*	5’- AGTGGTTAAGGACTTGAAACTG-3’
CRISPR RNA and HDR template for *GZMB* KI	CRISPR RNA: 5’- CATGAAACGCTACTAACTAC-3 HDR template: 5’-GGTGGCAGCGGTGGCTCAGGAGGTAGCATGGTCTCCAAGGGGGAAGAAGCCTCAGGCCGAGCCCTTTTTCAGTATCCCATGACAAGCAAGATTGAACTGAACGGCGAAATTAATGGAAAAAAGTTCAAGGTTGCTGGTGAAGGGTTCACTCCCTCCTCCGGTCGGTTTAATATGCACGCCTACTGCACGACGGGGGATCTCCCTATGTCATGGGTTGTCATCGCGAGCCCACTTCAATATGGATTTCACATGTTCGCCCACTACCCAGAGGACATTACACACTTCTTTCAGGAGTGCTTTCCAGGGAGCTACACCTTGGATAGAACCTTGAGAATGGAGGGGGATGGTACTTTGACAACACACCACGAATACAGCCTGGAGGACGGGTGTGTAACATCAAAGACCACGCTCAATGCCAGTGGATTCGACCCCAAGGGTGCTACTATGACTAAGTCATTTGTAAAGCAGCTTCCCAACGAGGTCAAAATCACACCGCATGGTCCCAATGGTATTCGCCTCACTAGTACGGTGTTGTATCTCAAAGAAGATGGCACTATCCAAATCGGGACTCAAGACTGTATCGTTACTCCCGTTGGAGGGAGAAAGGTGACTCAGCCAAAGGCCCATTTCCTCCACACCCAAATTATTCAAAAGAAAGATCCCAATGACACGAGAGATCATATTGTGCAGACGGAGCTGGCCGTCGCTGGGAACCTTTGGCACGGAATGGACGAGTTGTACAAG-3’.

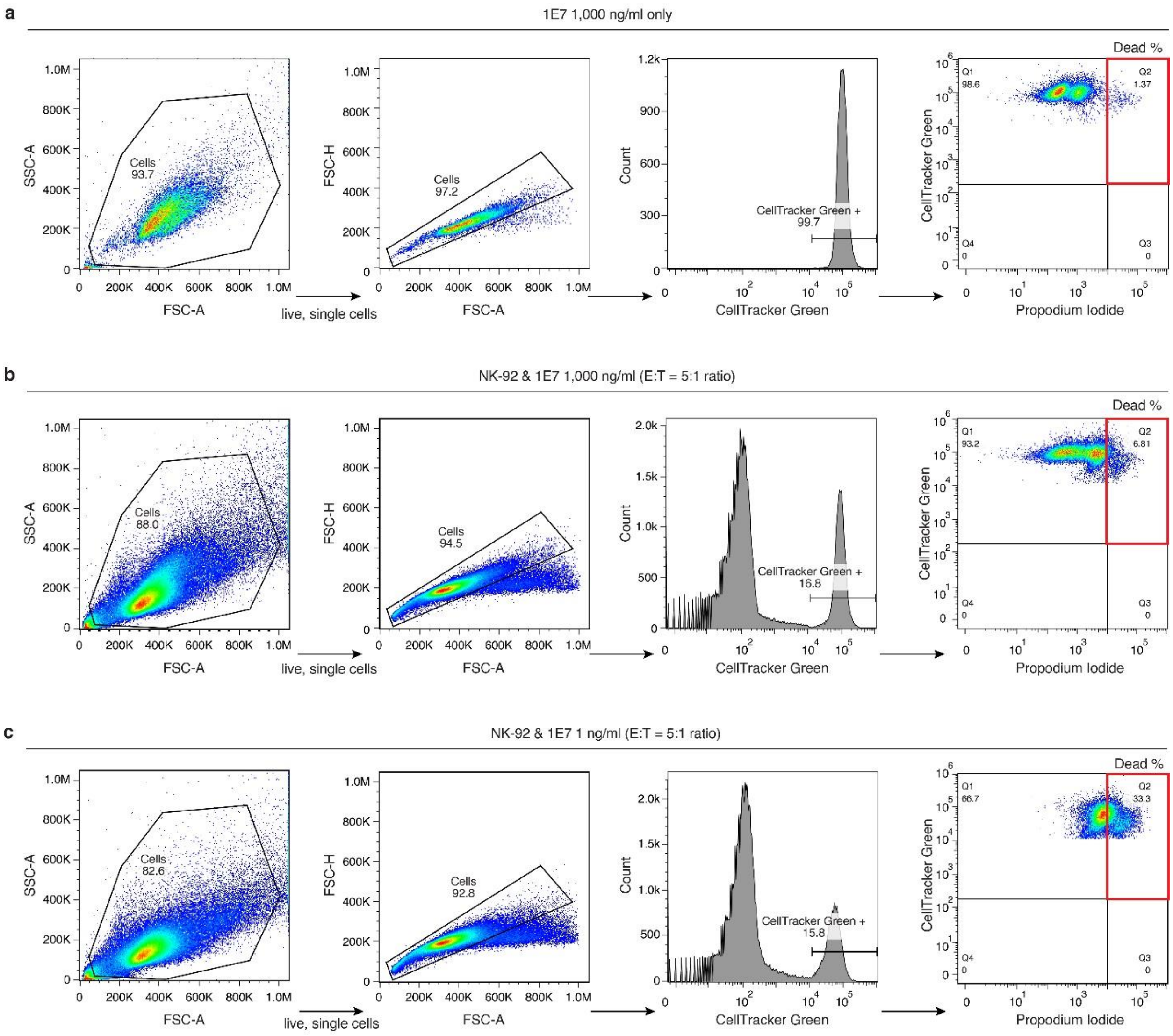
SUPPLEMENTARY FIGURE



Supplementary Figure 1. Flow cytometry gating strategies for Figures. Cells were first gated for size using the FSC-A/SSC-A gates, then single cells were selected (FSC-A/FSC-H). **a**, Gating on the selected cells for GFP nanobody-Alexa Fluor 647 (**Fig. 1d**). **b**, Gating on the selected cell for Muc1-JF549 (**Fig. 1i**). **c**, Gating on the selected cells for anti-CD19 Alexa Fluor 647 (**Fig. 3h**). **d**, Gating on the selected cells for anti-6X His-tag antibody with goat anti-rabbit IgG Alexa Fluor 647 secondary antibody (**Fig. 4b**). **f**, Gating on the selected cells for FluoTag-X2 anti-ALFA conjugated with Alexa Fluor 647 (**Fig. 5b**).



Supplementary Figure 2. Flow cytometry gating strategies for Extended Data Figures. Cells were first gated for size using the FSC-A/SSC-A gates, then single cells were selected (FSC-A/FSC-H). **a**, Gating on the selected cells for CD3-FITC, CD56 PE-Vio-770. Representative flow cytometry dot plots from 3 blood donors (**Extended Data Fig. 2a-b**). **b**, Gating on the selected cells for APC anti-human CD328 (Siglec-7) and mouse anti-human Siglec-9 with goat anti-mouse IgG Alexa Fluor 647 secondary antibody (**Extended Data Fig. 2c-d**). **c**, Gating on the selected cell for GFP nanobody-Alexa Fluor 647 (**Extended Data Fig. 3c**). **d**, Gating on the selected cells for NeutrAvidin Protein DyLight 650 conjugate (MAL-II), PNA-CF640R, and GFP nanobody-Alexa Fluor 647 (**Extended Data Fig. 4e)**. **e**, Gating on the selected cells for Muc1-GFP and GFP nanobody-Alexa Fluor 647 (**Extended Data Fig. 4i).**



Supplementary Figure 3. Flow cytometry gating strategies for NK-92 cell-mediated cytotoxicity. Representative flow cytometry dot plots: cells were first gated for size using the FSC-A/SSC-A gates, then single cells were selected (FSC-A/FSC-H). The cells then gated high CellTracker Green channel (BL1-A), then dead cell population were selected (Propidium Iodide: YL1-A/CellTracker Green: BL1-A). Target cells were 1E7 1,000 ng/ml only (**a**), 1E7 1,000 ng/ml with NK-92 cells (**b**), and 1E7 1 ng/ml with NK-92 cells (**c**). NK cell to target cell ratio is 5:1

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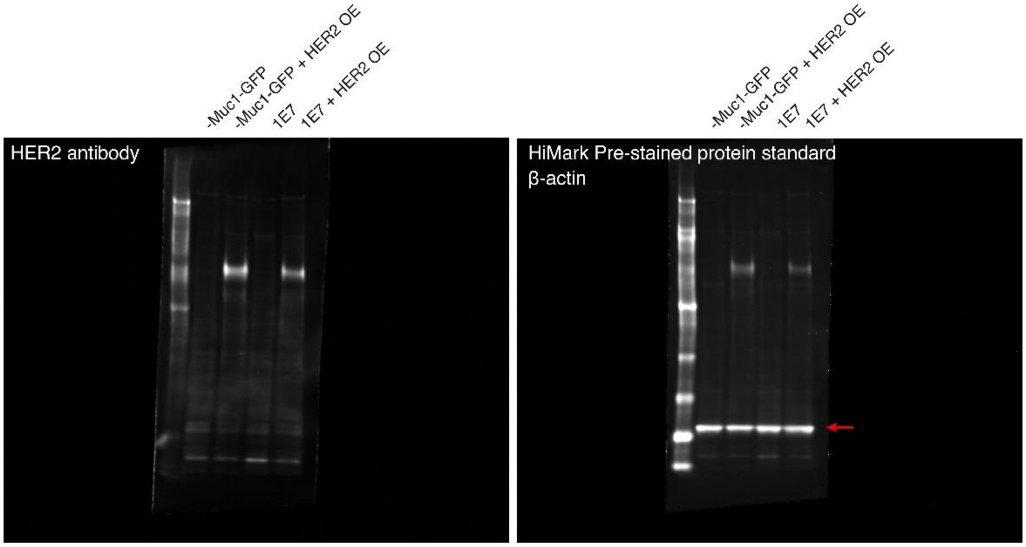
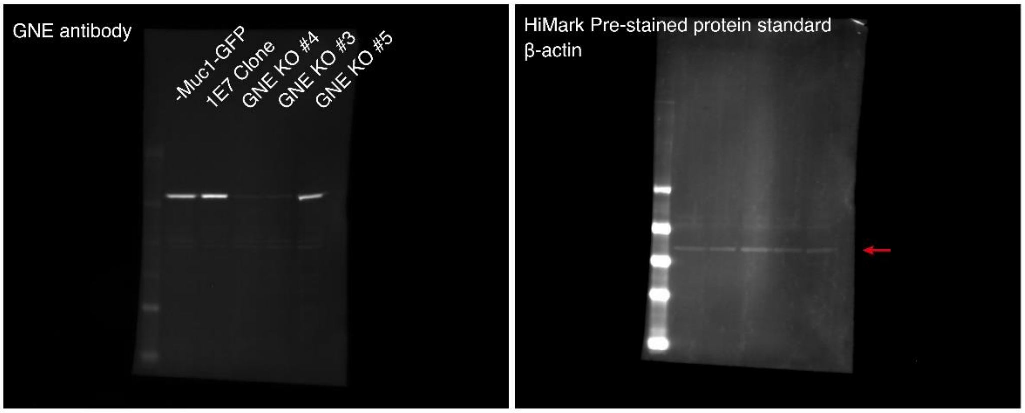
UNCROPPED IMAGES

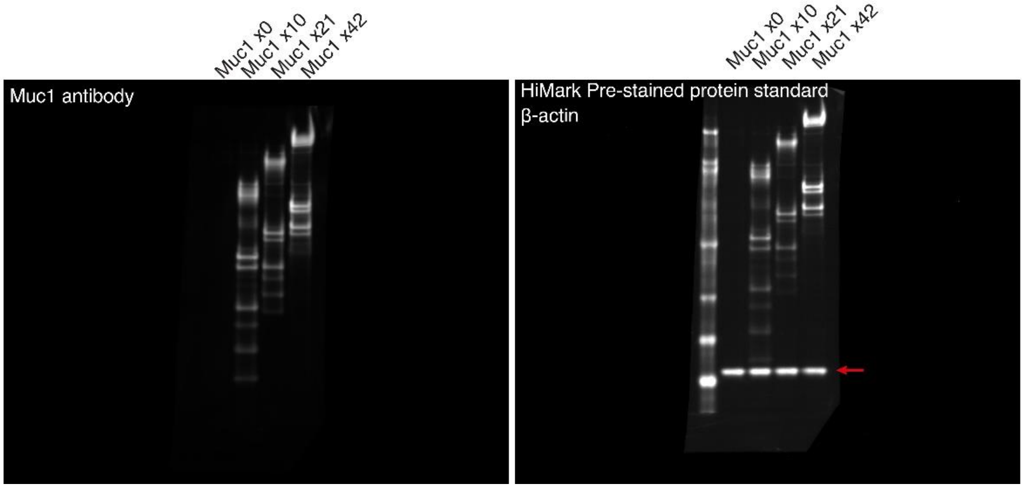
a Unmodified western image for Figure 3d



b Unmodified western image for Extended Data Figure 2i



c Unmodified western image for Extended Data Figure 3b



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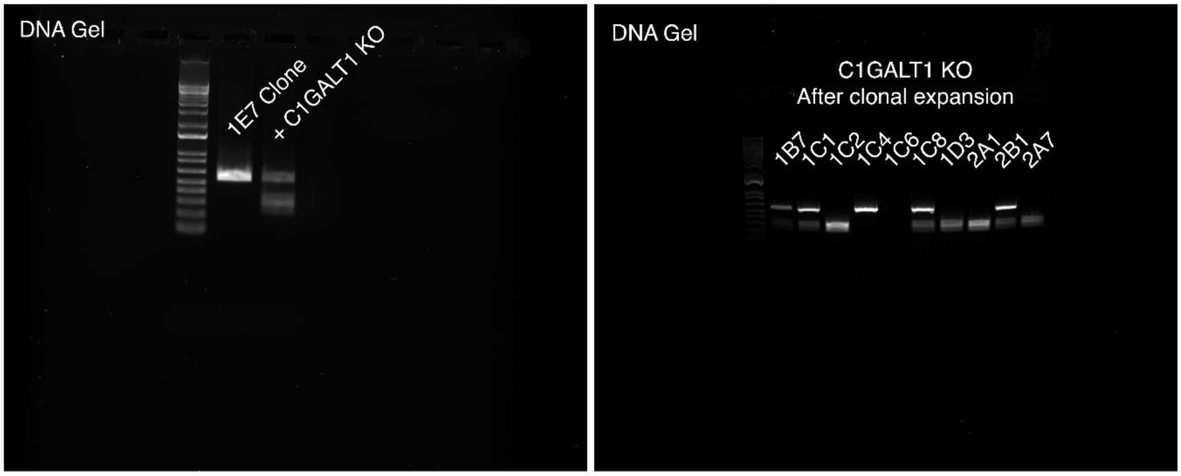
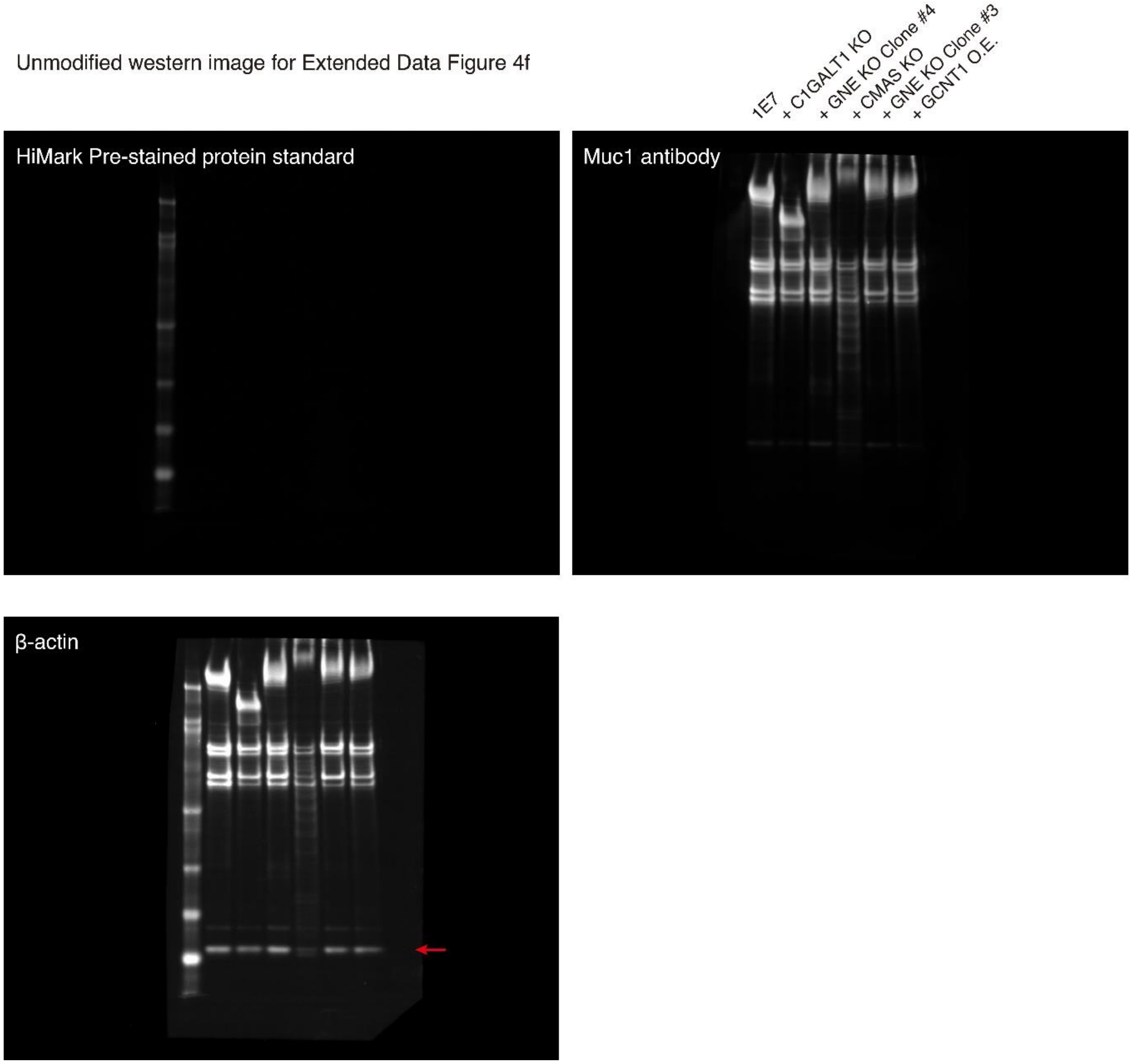


b Unmodified western image for Extended Data Figure 4f



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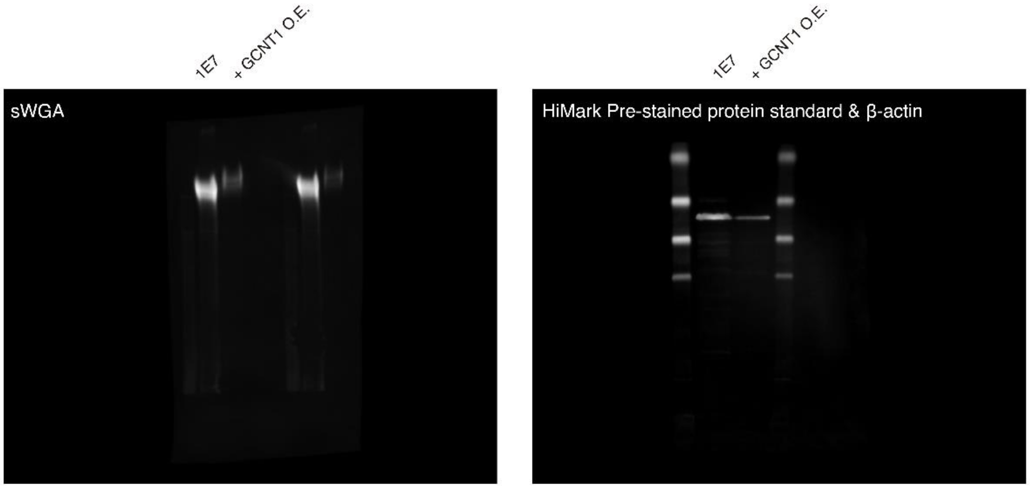

b Unmodified western image for Extended Data Figure 7e